



Volume 249

May 24, 1974

Portland problems . . .

BRITISH defence research has always been conducted in conditions of obsessive secrecy, and so the news that the Admiralty Underwater Weapons Establishment (AUWE) at Portland was to let selected guests in was a pleasant surprise. In 1968, the Select Committee on Science and Technology had urged wider dissemination of Portland's problems and achievements, and this was their belated response. Dr G. L. Hutchinson, the Director, indicated that he hoped that industry and universities would come into closer relationship with the establishment; he must also have had an eye on the defence review now being conducted. His budget is £9 million which he regarded as inadequate for some of his tasks.

The establishment is responsible for research and development for offensive and defensive naval requirements beneath the ocean surface. Within AUWE's orbit is torpedo homing, data collection in submarines, hydrodynamics, and all manner of other problems not all of which, doubtless, were on view; the greatest problem as far as one can tell is the antisubmarine warfare (ASW) requirement of finding submarines—in particular nuclear submarines—in the complex environment of the oceans. Sound in the kilocycle frequency range is the tool used but it is severely blunted by the sound velocity structure of the oceans which is neither laterally homogeneous, simple to parametrise, nor time invariant. The medium is barely conducive to propagation of sound over distances of more than a few tens of kilometres.

A first look at a new institution may be deceptive. Certainly security still poses enormous problems; one guesses from the unease with which some questions were answered or deflected that many would be glad when the whole thing was over and they could get back to work. There was an indication that open days would not become a regular feature of Portland's life; this, one hopes, is a sentiment which will be strongly resisted by the government, for the establishment has a lot to lose and nothing to gain by returning to obscurity.

The overall impression is somewhat sad. Amongst the exhibits were no surprises of the sort which mark out some laboratories as vigorous. Maybe there was more in what was not shown, but that is unlikely. The argument that the real stuff cannot be unveiled for reasons of security has always rung somewhat false and as likely as not means there is no 'real stuff'.

But there was more to the sadness than that. Many military laboratories all over the world were created in something like their present form in the ten years following the Second World War and recruitment was at its most vigorous at that time. They have all encountered the problems that Portland seems to have—of a now middle-aged professional staff with much experience (in this case of the hostile marine environment) but a diminishing capability for aggressive scientific thinking and yet insufficient other outlets. Universities offer teaching, committee work and administration. Industry

simply discards the nonproducers. Government scientific establishments can do none of this.

The results are two-fold. First, the creative ability declines as old solutions to problems are carefully refined whilst new solutions are not perceived rapidly enough. Second, research establishments become steadily less attractive to the brightest young graduates.

How far Portland conforms to this pattern is difficult to tell but all the symptoms are there. In addition, there is the resigned spirit so often manifested in government laboratories—the seeing of decisions as descending from 'our elders and betters' with the unambiguous indication that although elders not betters. There is also the horizontal compartmentalisation of problems—workers are not expected to know nor to take steps to know what is going on down the corridor. The compact rule of security, that the enquirer must not only be cleared but must also have a 'need to know', may cut down on spying but it reduces research creativity alarmingly. It is doubtful that many in Portland have access to enough pieces of, say, ASW research to put together a coherent up-to-date story of present abilities and future prospects. And yet any competent research student constrained from acquiring a global view of his subject would rightly walk out.

The problem of ASW is perhaps insoluble and it may be that this is our greatest guarantee of a measure of strategic stability for the next twenty years. On the other hand, there are many in the academic community who would like to become more involved in matters of defence—more, maybe, than the Ministry of Defence appreciates.

. . . Greenwich solutions

CHARLES II is said to have informed the Royal Society that he was "graciously pleased" with their work on useful problems such as finding the longitude at sea but not with their "childish diversions" such as weighing air. In the next hundred years, with the stimulus of a prize of £20,000, the art of navigation moved from complete ignorance about longitude to precise determination. It is a marvellous tale, always worth another hearing, and Eric Forbes' recent monograph (*The Birth of Navigational Science*, National Maritime Museum, Greenwich) is a good retelling because the role of Tobias Mayer and the method of lunar distances are fully explored.

Latitude is a relatively easy quantity to measure (so few look at the motions of celestial bodies these days that we make no apologies for an elementary exposition). The elevation of the Pole Star above the horizon at night is almost constant. During the day the maximum elevation of the sun also rapidly leads to the latitude. So with rudimentary devices latitude had been roughly determined since the sixteenth century. Longitude is altogether different, as some means of knowing both local time and some standard time, such as Greenwich, is needed. Local time is relatively easy to obtain from midday, and even quite inaccurate clocks can be used with daily corrections. Greenwich time is an altogether different proposition. So serious was the problem to mariners that large cash prizes were offered for its solution by the governments of France, Holland, Spain and Venice in the sixteenth and seventeenth centuries. As early as 1530 two distinct methods had been proposed: one that a high quality clock be carried on a ship, to take Greenwich time along, as it were; the



other that a celestial clock be used such as the steady progression of the Moon amongst the stars.

The two most promising methods—superior clocks and so-called lunar distances—were still far from accurate enough when the British government set up the Board of Longitude in 1714. Standard practice on long voyages was to run down lines of latitude into one's destination, a potentially catastrophic procedure if sailing to an island and unsure whether east or west of it. As late as 1741 Anson in the *Centurion* lost eighty men of scurvy in the twelve days he spent hunting for Juan Fernandez (of course not only were ships often misplaced, so, occasionally, were islands). The Board offered a results-related award—£20,000 for an accuracy of half a degree in longitude at the end of a six-week voyage to the West Indies, £10,000 for an accuracy of a degree. Sir Isaac Newton summarised the problems in making an accurate enough timekeeper—"the Motion of a Ship, the Variation of Heat and Cold, Wet and Dry, and the Difference of Gravity in different Latitudes". An accuracy of about two minutes would be needed at the end of the prescribed sea voyage for a half-degree accuracy, and clearly no pendulum clock would be up to that. Spring-driven clocks had been made as early as 1500 but by 1714 had never been compensated properly for temperature. Lunar distances might take the prize.

On the practical side one had to measure the angular distance between the moon and some prominent zodiacal star. This changes 13° in every 24 hours, so the invention in the 1720s of the reflecting octant with an angular accuracy of $1'$ implied that if the lunar motion were perfectly tabulated, time by the Moon could be accurate to about two minutes once atmospheric refraction and geocentric parallax were allowed for. Two minutes of time is half a degree of longitude so the prize was in sight, at least if the tables could be perfected. It was to this that Tobias Mayer of Nuremberg and Göttingen most successfully devoted fifteen years from 1747.

Mayer was a cartographer and his interest in astronomical determinations of longitude came not from any nautical experience (he never even saw the sea) but from a desire to improve land surveying in Germany. He realised as he searched for higher accuracy that existing lunar data was unreliable and even that some of the stellar positions were unsatisfactory. Having read Euler on analysis and mechanics he ventured into the theory of the Moon's motion around a spheroidal Earth.

His first tables of the motions of the Moon in 1752 were criticised for discrepancies and for a certain inscrutability but by 1754 he had shown that the discrepancies were in contemporary star tables, and the usefulness of the tables for longitude determination was demonstrated. Euler now persuaded him to go for the prize money which he did in a Memorial to the Lords of the Admiralty beginning: "Having by indefatigable application and a great deal of trouble found out the longitude at sea . . ."

His work was well received in London, but the Board of Longitude delayed and Mayer died before a decision was made. Nevil Maskelyne, Astronomer Royal, used the tables with success on a voyage to St Helena in 1761 to observe a transit of Venus, and there was general agreement that Mayer's widow merited the £10,000 prize. The Board thought otherwise (they were probably somewhat embarrassed by their simultaneous encourage-

ment of Harrison and his chronometers) and eventually awarded her only £3,000. This grudging acknowledgement did not inhibit the Commissioners of Longitude from using Tobias Mayer's tables to generate the first Nautical Almanac for 1767. But now the potentially more accurate chronometer, untied to problems of the Earth's rotation and the Moon's complex orbit, began to come to the fore.

John Harrison (1693–1776) stands out as a clock-maker head and shoulders above his contemporaries. He came to London in 1728 with two inventions which were to start him on a lifetime of battling with temperature fluctuations and poor lubrication—the 'gridiron' pendulum of brass and steel rods and the 'grasshopper' escapement which required no oiling. His first clock, H1, was tested so effectively on a voyage home from Lisbon in 1736 that Harrison, having estimated the position one and a half degrees west of the calculation by dead reckoning was proved right on landfall.

The money was not that easily won. The Board helped Harrison financially, but the Spanish wars and his own self criticism prevented a direct assault being made on the prize until 1761 by which time Harrison had built two other chronometers with which he was not totally satisfied, and now produced the watch H4, a mere five inches across. H4, surely the finest watch ever, was taken to Jamaica in an inconclusive trial, but in 1763 was tested again and showed an error of less than one tenth of a second per day. This should have brought Harrison his £20,000. The Board paid half but demanded that he secure his second £10,000 by showing that H4 could be made by others. Eventually George III exerted pressure and in 1773 Harrison received another £8,750.

Go to Greenwich to see Harrison's chronometers. They are displayed in a serene room almost inaccessible to the casual visitor like the Lady Chapel of some cathedral. The display is superb, as the big clanking clocks come on you one by one down the room. And then suddenly there is a small ticking watch, H4. The atmosphere is almost religious, and the visual surprise will live with you for a long time.

100 years ago



Colonel Gordon left Khartoum on March 21, and in his last letter from Fashoda, 10° N., he touches on some of the scenes on the banks of the river—the storks, which he was in the habit of seeing arrive on the Danube in April, laying back their heads between their wings and clapping their backs in joy at their return to their old nests on the houses, now wild and amongst the crocodiles 2,000 miles away from Turkey; the monkeys coming down to drink at the edge of the river, with their long tails, like swords, standing stiff up over their backs; the hippos and the crocodiles. Such scenes to a lover of nature, as Col. Gordon is, doubtless would serve to make up in some measure for the loss of civilised society and comforts.

Does cancer research have realistic aims?

The Imperial Cancer Research Fund tries with its 1973 report, recently published, to appeal to a wider audience. Brian Ford takes a look at the report and at broader issues in cancer research.

CANCER research is a blend, not altogether a happy one, of the assured with the uncertain, the esoteric with the mundane; yet in recent years some remarkable improvements in survival rates have been achieved in fields that include breast cancer, leukaemia and the lymphomas. That alone is sufficient to refute allegations that this speciality is uniquely able to consume public funding for little tangible benefit. Successes in specific areas are, however, not themselves a guarantee that priorities are correct or that courses of action have been chosen for valid criteria. The latest report of the Imperial Cancer Research Fund (Lincoln's Inn Fields, London WC2) throws an interesting light on the advancing front of progress, and mirrors some deficiencies in the philosophy that underlies it.

Past reports have been issued by the fund in a different form. As the Foreword acknowledges, they have been an unsatisfactory mixture of facts, figures and data presented (particularly where clinical findings and research are concerned) in the specialist form least likely to attract interest or even understanding from the outside reader. The survey for 1973 is therefore novel in approach, for it embraces three specific and distinct areas of interest: the accounts (which are bound as a separate volume), the research reports as such and the Director's assessment. There has been a deliberate attempt to word this introductory section carefully so that it may be assimilable to the intelligent outsider (who is described as a 'non-scientist' by the Foreword, but in this era of isolationist specialisation is as likely to be a psychologist or physicist) and Michael Stoker has taken the brave step of using his easy manner and flow of commonplace English to accomplish the task. For so long has it been widely assumed that understandable terminology and uncomplicated syntax are indicators of superficiality in approach, that this step is perhaps doubly notable. Yet in some respects, by casting a more direct light on the state of the art, it is a less than satisfactory exercise.

He draws a close parallel between

the immune response that may be invoked against malignant cells and that seem to act "against measles". Similarly far-fetched analogies are becoming popular (indeed the Nature-Times News Service report dated April 22, 1974, draws a similar parallel) yet it is arguably unrealistic. Surely the infective invasion of susceptible tissues by a transmissible viral genome is operationally distinct from neoplastic transformation, and the quest for a genotypically polyvalent vaccine, as it were, must be a different kettle of fish from the development of measles immunisation. One looks back to the trial-and-error attempts of Jenner to experiment with vaccinia and Pasteur with rabies, both by good fortune working with material which responded uniquely to their techniques though neither had much reason to suppose it would do so; and one assumes there is something more predictable, less metaphysical, about modern medical methodology. Yet here we are, in the 1970s, with electroshock in the psychiatric hospitals and vaccine-shock in oncology, hopefully injecting BCG or irradiated leukaemic blast cells, in the hope of gingering up the immunological sensitivities of a cancer patient.

In Stoker's words it looks sound enough: "The vaccine is made from leukaemic blood from another patient, which is slightly different from the recipient's own blood and may, therefore, give an additional boost to the defences". Yet it is this very clarity which reveals the extent to which our

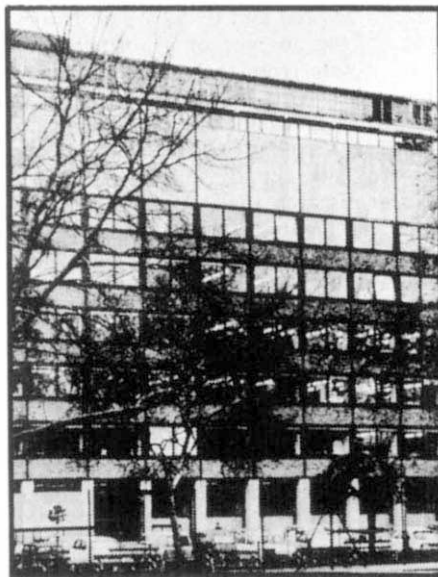
knowledge is clouded and incomplete.

There are many other fields of investigation in which the dearth of detailed knowledge, and the inadequacy of many orthodox beliefs, is demonstrated. The report mentions that Rubens and Dulbecco have investigated the use of cytotoxic compounds adsorbed onto tumour-specific antibodies, the principle being to utilise the antibody as a vehicle for the drug, only to find that the loose covalent association that was assumed did not, apparently, occur in their trials. It now seems that the increase in susceptibility of the transformed cells is coincidentally induced by the presence of antibody; how remains uncertain.

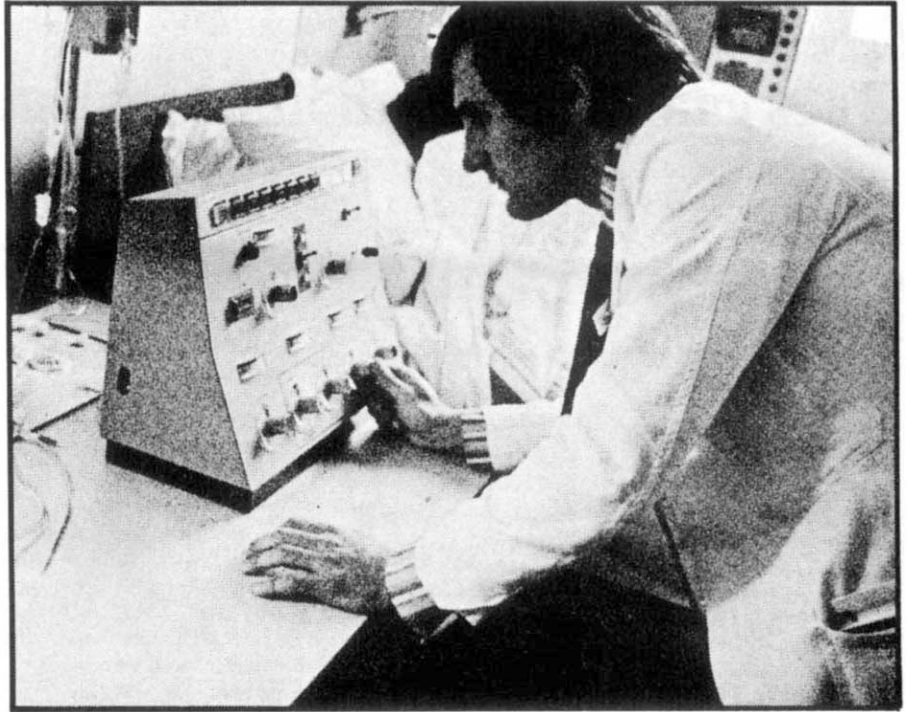
Though some of the practical problems of cancer management remain poorly understood, the study of what we might in the broad sense term oncogenetics is inspiring. So too is the interest in conceptualising about cell growth. I believe it is helpful to construct greater models by which to account for the lesser datum; holistic concepts through which to marry reductionist dogmas. In this respect it is heartening to see the work which Martin and his colleagues have under way, which construct novel interpretations of the dynamic status of mature cell communities; models that are not profound and whose principal benefit lies in their being far from complex, but which provide an intellectual synthesis of observed phenomena.

Growth-rate regulatory mechanisms are what cancer is all about, and it is fitting that one of the leading ICRF groups should be the Department of Cell Regulation. Yet, when our understanding of these mechanisms is so profoundly complex in some respects, how surprising it is that Stoker can demonstrate the simple fact that boundary layer gradients may be as important as cell-to-cell contact in regulating cell growth rates. This contrast between the detail of our understanding in some respects, and our ignorance of far more fundamental, practical issues in others, can be illustrated by other reports in the document. Though it is now beginning to seem possible that we may be able to screen urine samples as a means of detecting those patients peculiarly susceptible to breast cancer, it is still not known whether radical mastectomy or wide excision are preferable in the early stages of the disease. Here too we see progressive developments in one aspect of a discipline

ICRF headquarters in London



Research in progress at St Bartholomew's Hospital, London, where the Imperial Cancer Research Fund established a Professorial Chair in Medical Oncology in 1971.



contrasting with ignorance of a seemingly elementary nature in another.

In certain respects the sense of imbalance that parts of the ICRF report engender are no more than a mirror of the greater problems faced by modern science. To rely, in some primitive Baconian sense, on observing phenomena and then adopting the correctly self-critical mode advocated by the followers of Professor Popper, seems to be the only true path to objectivity and insight. Too often it tends, instead, to blind one to the true relationships between the larger fragments of science, and it conveniently ignores the weaknesses and foibles of human nature. I believe we should now recognise the inability of modern scientific research to fulfil its function adequately by relying solely on what one might term inspirational research. Perhaps we need something of what the Americans would term the 'project' approach, in which teams or individuals are asked to prosecute a given line of investigation which fitted into the whole.

The argument against this is that in this way we tend towards the dictatorial direction of research. What we seem to forget is that research is being surely directed at the moment, but directed by whim, by fancy, by inclination; it is directed by current trends, by the consensus paradigm subconsciously obeyed by an introspective discipline of science; and it is directed by the distribution of funds and support. Though there is no need to dictate science policy, or to set the sights of science at some invisible target, there is certainly room for a little calculated realism somewhere in the mix.

Even though we pay little attention to the criteria by which our research priorities are selected, they certainly exist. To utilise a framework of agreed values to supplement—though in no sense replace—'personal fulfilment' guidelines is more likely to bring us nearer the truth than shots in the dark, no matter how inspired the marksman. Though it is undeniably true that free-wheeling

chance gave us *Penicillium notatum* and the earlier discovery of antibiotics, it was the directed research of wartime that gave the world penicillin and the antibiotic age. In an area such as cancer research, where the main concern of all is to bring about an increase in knowledge, unless they or their relatives are suffering from the disease, when the prime requirement is seen as the saving of life; here more than anywhere we need guidance by consensus and objective-setting as a supplement to inspiration.

To speak of cost-effectiveness in research is irksome—until we realise how costly it is, and how badly civilisation needs the effects.

If we have to pick out one example of an individual revelation from the ICRF report, there can be no better example than the discovery of a demonstrable difference between the cell surface in normal and transformed tissues, namely the absence of a normally-present protein from the surface of malignant cells. This has been the work of Richard Hynes in the Department of Tumour Virology, and is a sound and hopeful advance. Indeed it is in virological research that the ICRF has perhaps its greatest claim to fame, and around which at least four of the groups base their work. Yet man seems to show few signs of suffering from virus-induced tumours, and current estimates suggest that 60–90% of all malignancies in man are caused by chemical carcinogens. Should one suggest that—if resources are limited—more attention should be paid to the practical problems of patient care in hospitals and at home?

We still know little about cells in communities. A model of histological

or developmental normality—cell sociology, one might say—is at least as important in understanding malignancy as would be a comprehensive understanding of a single cell. If we are to promulgate pure research, ought it not to concern itself with relatively unexplored avenues such as these?

It is often said that in cancer one cannot experiment on humans. But one does, of course, perpetually in a manner which (though entirely justifiable and ethical) must surely seem incongruous alongside specialised research in other fields. How is it there is still the scent of alchemy in the air where much chemotherapy is concerned, utilising drugs of high potency but uncertain action in an empirical and almost haphazard manner? Should the arm be immobilised after breast surgery or allowed freedom of movement as an aid to fluid drainage? Are steroids helpful in the therapy of malignant disease? These questions (which seem fundamental indeed alongside specialised virus research) are only now being resolved, by experimental trial.

When there are many short term, immediate questions to be answered, is it entirely justifiable to press ahead in such detail on animal research based on cancer models that are not apparent in mankind? Perhaps we should take heed of the comments in last year's report from the International Agency for Research on Cancer in Lyon concerning the near impossibility of extrapolating the mass of data from animals to man. Indeed, is not the problem of cancer research that we know too much: we have too many data and too few conceptual pegs on which to hang them? And is the balance of cancer research entirely fair to the patient?

international news

THE prospects for international control of chemical weapons could well hang on the results of an intensive review of the United States chemical warfare policy now being conducted by the Nixon administration. It is the first such review for nearly five years.

Begun quietly by the National Security Council (NSC) in December last year, the review is a two-pronged study, first of an impasse which for four years has prevented the United States from ratifying the most important chemical weapons treaty ever negotiated—the so-called Geneva Protocol of 1925, which bans the first use of chemical weapons in war. And, at the same time, the NSC is taking a close look at the Pentagon's plans to modernise its existing nerve gas stockpiles by replacing them with a new generation of chemical weapons called binaries. According to Administration spokesmen who testified on nerve gas policies before a congressional subcommittee earlier this month, the review should be completed in the next few months.

Meanwhile, Congress is also faced with an important decision in the chemical warfare area, for the Pentagon has already requested approval of funds to begin producing binary weapons at the Pine Bluff arsenal in Arkansas. A decision must be made this summer on whether or not those funds will be approved.

These reviews and decisions are taking place against a backdrop of international negotiations being conducted in Geneva under the auspices of the 26-nation conference of the Committee on Disarmament (CCD), the ultimate aim of which is to negotiate a treaty banning not only the use of chemical weapons in war but also their production and stockpiling. In other words, the goal is complete worldwide disarmament. What happens in Washington in the next few months will be crucial to the outcome of those negotiations.

At present, the official Administration policy on chemical warfare, as outlined by President Nixon in a statement in November 1969, is that the United States will never be the first to use chemical weapons in a war, and that it has renounced entirely the use, production and stockpiling of bacteriological weapons. Coupled with the no-first-use policy, however, is the understanding that in the absence of worldwide chemical disarmament, the

US at chemical war crossroads

Colin Norman, Washington

United States army needs to maintain extensive stockpiles of nerve gases to deter other countries from initiating chemical warfare or, if deterrence fails, so that the United States can retaliate in kind. For those reasons, some 45 million pounds of nerve gases are now stockpiled in bases throughout the United States, in Johnston Island in the Pacific, and at one location in Germany.

Unfortunately, however, since the United States has never ratified the Geneva Protocol the policy of no first use of chemical weapons is nothing more than a promise, and until the protocol is ratified, United States intentions will never have the force of international law. At present, ratification is bogged down in a dispute between the Administration and the Senate Foreign Relations Committee over the Administration's interpretation of the terms of the protocol. It will be crucial to the outcome of the CCD talks that this impasse be broken.

The nub of the matter is that when President Nixon submitted the protocol to the Senate for approval in 1970, he did so with the express understanding that riot control agents and herbicides are not included in its provisions. In other words, even if the United States government formally ratifies the protocol it would still consider itself free to use those agents in warfare—in fact the United States army was doing just that in Vietnam at the time Nixon submitted the protocol to the Senate. The Senate Foreign Relations Committee argued, however, that the protocol should include all chemical weapons and it is refusing to put the matter to a vote in the full Senate until the Administration backs down from its interpretation.

There the issue has rested for the past four years, but at least the NSC is now taking a close look at the Administration's position. Whether a complete change of mind will ensue from the review is, however, doubtful to judge by statements made by Administration officials who testified before a subcommittee of the House Foreign Affairs Committee earlier

this month. Mr Amos A. Jordan, Assistant Secretary for Defense for International Security, argued that herbicides and riot control agents played a valuable and "humane" role in the Vietnam war, for example, and he said that the Administration's position has not changed.

Failure to ratify the Geneva Protocol is understandably viewed within the CCD talks as a severe stumbling block in the path of efforts to secure a more extensive treaty which would outlaw chemical weapons entirely. Furthermore, a treaty outlawing the production, use and stockpiling of biological weapons, which was negotiated at the CCD talks a couple of years ago, is also held up in the Senate Foreign Relations Committee awaiting a final resolution of the Geneva Protocol dispute.

That is bad enough, but according to a number of observers, the Pentagon's plans to develop binary weapons could eventually kill the CCD talks entirely. Binary weapons have been under development by the United States army for the past ten years or more, but they are now coming up for a number of crucial decisions. In short, the weapons consist of two "relatively non-toxic" chemical components which form a lethal nerve gas when they are mixed together. The idea is that they would be kept apart until they are required for use, and they would then be fed into separate compartments in a shell and allowed to mix only when the shell is safely on its way to the target.

The Pentagon's argument for binaries is simple enough. Since the weapons are safe to store, produce and transport, they form ideal replacements for the existing ageing stockpiles of nerve gases. According to official army statements, nerve gas munitions have shelf life of only 15 years, and thus existing stock of filled weapons will have to be replaced by about 1985. Consequently, in September last year, the army began to tout the advantages of binary weapons in public statements and in congressional testimony, and it has now asked Congress to approve a request for \$5.8 million to begin production of 155-mm binary-equipped artillery shells. That request is now under consideration—in secret—by a Congressional Appropriations Committee: it is being hinted that the request is running into some trouble.

The binary programme is being opposed by a small number of congressmen concerned chiefly over the possibly damaging effects of a massive new chemical weapons programme on the Geneva CCD talks, and they seem to be picking up some influential support from a few conservatives connected with military affairs—a sign, perhaps, of the way the wind is blowing, is that the House Armed Services Committee recently voted to cut about \$1.9 million from the Pentagon's request for research and development related to binaries (this, it should be noted, would be an entirely separate demand from the demand for production funds).

One particularly effective argument, which surfaced during the House Foreign Affairs Committee hearing earlier this month, is that the Administration is now conducting an "urgent review" of the binary programme itself, and that Congress should therefore delay approving the Pentagon's request for production funding, at least until the National Security Council has decided whether or not the Administration is actually in favour of the programme. At present the Congress is placed in the position of deciding whether or not to provide money for a programme which may be killed off by executive fiat at some future date—a situation that would arise only in the highly sensitive region of defence spending, and which gives substance, to the view that there is little executive scrutiny of the Pentagon's annual demands before their submission to the Congress.

Although that argument may ultimately carry most weight in the Congress, it became clear during the House hearings that the binary programme could well be in trouble for a variety of other reasons, and that there is a division of opinion actually within the Administration on the merits of pushing ahead with a chemical weapons programme of any variety at the current stage of the Geneva talks. The NSC review of the binary programme will inevitably be subjected to some critical 'input' from the Arms Control and Disarmament Agency, which has admitted serious disquiet over the possibility of a probinary decision damaging—perhaps fatally—the CCD discussions. Dr Fred Ikle, Director of ACDA for the past year, said, under heavy questioning from the committee chairman last week that: "It is my judgment, based on arms control considerations—and this is a personal view—that at this time the pros and cons come out in favour of not going into production (of binaries)".

The military view, as expressed by Mr Jordan, is that present nerve gas

stockpiles "do not fully provide the capability we believe is necessary to adequately support all United States forces in case chemical warfare is used against us. Binaries," he said, "provide significant operational and safety advantages over any other known approach which could have been selected for modernisation."

The situation is therefore the familiar one of the official United States negotiating position being in favour of disarmament, yet at the same time the Pentagon is pushing for a new weapons programme. According to Representative Patricia Schroeder, who attended the CCD talks last year as an official United States observer, "Where many nations last summer simply regarded the United States as having assigned a rather low priority to CCD activities, by mid-March, as the 26 reconvened, one of the more influential western delegates, Dr Alfonso Garcia Robles, leader of the Mexican delegation, went so far as to suggest that we plan to trigger a chemical arms race."

Another factor which has recently been injected into the binary debate—and one which could ultimately prove decisive—is the potential opposition of

Western European Governments to the programme. In fact, Mr Leon Sloss, Assistant Secretary of State for Political-military Affairs, admitted under questioning by the House Foreign Affairs Committee that the binary programme is running into some diplomatic difficulties.

The reason is simple. Binary weapons make sense only if they are deployed in forward positions, ready to retaliate against an aggressor who initiates chemical warfare. In other words, nerve gases stored in depots in the United States would be of little use as quick response weapons against the Soviet Union, and the only place it makes sense to keep the weapons is in Europe. Sloss refused to discuss the subject in open session, but admitted that any attempt to expand nerve gas stocks in Western Europe would run into difficulty.

Some British observers feel that the outcome of all this will be that the Administration will decide that the programme is not worth the diplomatic storm that it may cause and that it will eventually be stopped. But sources on Capitol Hill, while remaining moderately optimistic, believe that the issue is far from decided.

Another refusal to join the NAS

FOR most American scientists, election to membership of the National Academy of Sciences (NAS) is regarded as an accolade second only, perhaps, to being awarded the Nobel Prize. But during the past four years, two scientists have resigned from the august body, and two others have refused to accept membership when it was offered to them. The chief complaint all four have raised is the academy's close involvement with the research programmes of the Department of Defense.

The latest to decline association with the academy is Richard Levins, a mathematical biologist from the University of Chicago, who was elected to the Academy at the general meeting in April. Levins turned down the offer in a letter dated May 10, stating that his "first and most urgent concern is the continuing participation of the academy in military matters".

Levins also sharply criticised the policies of the academy's President, Philip Handler, but he argued that the organisation's connections with the military are "not the result of a perversion of the academy's nature by Phillip [*sic*] Handler or his predecessors, but of a faithful interpretation of its charter and traditions".

The academy's charter, received from President Abraham Lincoln, specifically gives it the role of advising the government, and Levins therefore

argues that it would be self defeating to accept membership in the academy and then try to reform from the inside. His letter states: "I cannot hope to remedy this situation by planning with other colleagues to replace Mr Handler with a more liberal president, or by manoeuvring to restore the NAS to its true mission: it is performing its true mission, and I find that mission repugnant."

Levins specifically attacks Handler for trying to "weaken any criticisms of the military, as he did in his covering letter (to the academy's report on herbicide damage in Vietnam), where among other things he dismisses the evidence of damage to human health and of death caused by herbicides."

For his part, Handler claims that the amount of classified work undertaken by the academy has declined during the past few years. He stated in his annual report to the membership that about 5% of the funds funnelled through the academy this year were concerned with that resulted in the printing of classified reports.

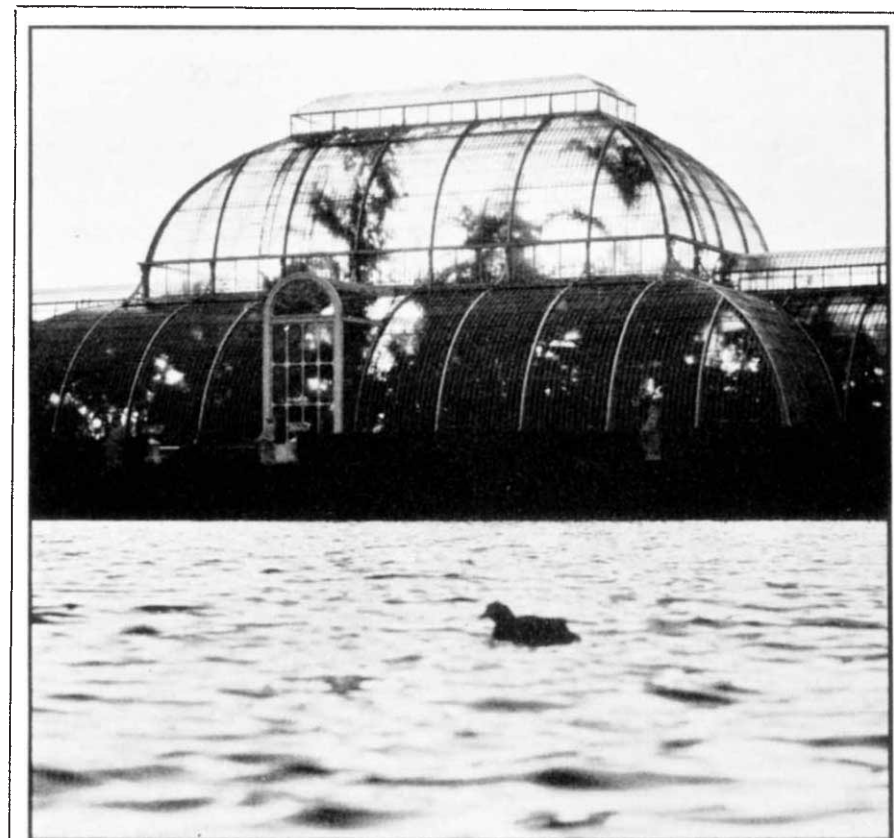
Be that as it may, Levins and others who have attacked the academy for its association with the military would like to see the organisation completely sever its ties with the Department of Defense. In refusing to accept membership of the academy, Levins is following a precedent set by Dr George Field, a Harvard astronomer.

State of the world: IISS reports

"ALMOST medieval turbulence under a nuclear umbrella", is how the International Institute of Strategic Studies' (IISS) annual report for 1973 describes the military and political scene last year (IISS, 18 Adam Street, London WC2). In a year marked by the withdrawal of the United States from Vietnam, a commitment between President Nixon and Secretary Brezhnev to negotiate a SALT II agreement, the downthrow of President Allende, the Middle East War, the appearance of the oil weapon and ominous creakings in unity not only within Europe but with the United States, the epithet is well deserved.

The greatest strategic development was undoubtedly the use of oil as a weapon which "opened up prospects of entirely new political balances". The quadrupling within a year of oil prices led to Dr Kissinger's proposal for an Energy Action Group, but there were many defections from the united front that was hoped for as western Europe and Japan pursued bilateral deals with oil producers; the ingredients of many of these included arms sales. Already in the eighteen months prior to the October war nearly \$5,000 million worth of arms deals had been made with Persian Gulf states—mainly Iran and Saudi Arabia. Iran, in particular, sees threats both internal and external—indeed there are potential dangers on all sides except from Pakistan. The IISS finds it difficult to predict the military future in the Gulf, so complex is the scene. On the one hand armed-to-the-teeth means bloody conflict; on the other, the strength of Iran and Saudi Arabia means stability. A familiar problem for strategists.

The prospects for SALT II are gloomy, as United States-Soviet tensions grew in 1973. There are some sticky problems for negotiators. In August the United States announced MIRV tests had begun in the Soviet Union thus starting the process by which American qualitative superiority though with numerical inferiority in missiles is eroded. And ultimately the accuracies of land-based missiles are so great that if the number of such missiles possessed by one side exceeds that of the other, first strike by the bigger force against the other side's launchers as good as ensures missile victory. The United States is believed to be aiming in SALT II for some sort of numerical equality with limited freedom to mix missiles. This, of course, leaves the American bomber superiority untouched. The Soviet Union is said to have responded that numerical equality would require the dismantling of American Forward-based Systems such



Kew: the Palm House

VISITORS had an opportunity to see the other side of Kew when the Royal Botanic Gardens held its open days recently, and the laboratories, library and herbarium were open to the public. The Director, Professor J. Heslop-Harrison, stressed the importance of the gardens as a repository for living wild plant species. The plant kingdom is in retreat, he said, and about a tenth of known angiosperm species are in grave danger. By the end of the century half of these would almost certainly be extinct. As part of a worldwide effort to conserve this fast disappearing genetic resource, a seed bank has already been set up which he hopes will eventually form the core of a national seed bank comparable with those in Leningrad and Colorado. Equally as disastrous as the

disappearance of individual species is the destruction of large areas of natural habitat by man's expansion. Professor Heslop-Harrison said that although the general public is now sympathetic to the idea of wildlife conservation the less glamorous concept of vegetation conservation is difficult to communicate, although of prime importance. If you want to save the tiger, he said, first save the teak forests in which it lives. In the gardens themselves, the Temperate House, designed by Decimus Burton and built between 1860 and the 1890s is now being completely restored and renovated as was the Palm House some years ago.

as European submarine bases. She also proposed a prohibition on further research and development of bombers, cruise missiles, penetration aids and air-to-surface nuclear missiles. "Outrageous", said the United States.

The problem for the 1990s is the submarines, the institute believes. Much will hang on breakthroughs, if any, in anti-submarine warfare technology, and there are various actions that could be taken to ensure submarine invulnerability. Continuous tracking of submarines could be prohibited, as could large scale sonar installations. Sanctuaries could be provided for submarines. This is the stuff of arms control in the

late 1970s and the sooner it is widely discussed the better.

Will the Soviet Union attack China in 1974? The IISS sees this as a serious option for Soviet policy, but concludes that the days are over when a surgical strike against Chinese nuclear facilities would have destroyed the country's weapons programme. Further, China now has more allies, and is capable of inflicting some damage on Soviet cities.

The troubles of Angola, Mozambique, Rhodesia, Namibia and South Africa with insurgents are given four pages, the Northern Ireland troubles not a word. A pity; a brief analysis in the IISS's lucid style would have helped.

EUROPEAN manufacturers of PVC are to spend at least £4 million on major plant modification where workers are liable to come into contact with vinyl chloride monomer (VCM). The move follows reports earlier this year that exposure to VCM had caused the deaths of plastics workers in the UK and the United States. Subsequent enquiries have revealed 18 cases of liver cancer among PVC workers—12 in the United States, one in Britain and possibly five in Europe. Ironically, the very rarity of the angiosarcoma involved has allowed researchers to say so concretely that it was caused in the occupational environment. Since no other chemical agent is known to produce angiosarcoma so directly it is difficult for the industry's scientists to look for outside causes, whereas a common or garden lung cancer might easily have been attributed to any number of external factors.

The Chemical Industries Association (CIA), which represents the big four British plastics producers involved in the affair, is at great pains to point out the highly moral way in which the companies have responded to the news that VCM is a bad smell in a production process which looks like a bed of roses profit-wise. Not only did the manufacturers sponsor Maltoni's Bologna programme (which put the finger on VCM) but having found themselves on the spot when the unpleasant tidings broke earlier this year they have in the space of three months reduced airborne VCM concentrations from 150ppm to 50ppm in polymerisation plants, and to about 5ppm on the actual VCM production line. And this when the legal limit stands at 200ppm. It should be noted on the other hand that the 200ppm limit existed simply as an anti fire and explosion safeguard before anybody had any inkling

CIA comes clean, almost

John Hall

that the stuff was no good for the liver.

In those days, ladies were spraying their curls with lacquer carried on pressurised jets of VCM from candy-coloured aerosols, nobody turned a hair at the thought of orange pop coming home in PVC bottles and an American liquor company was even planning to market the hard stuff in PVC packaged doses. They ran a programme just to see if alcohol was affected by contact with the plastic, and the answer was that it was OK except that it seemed to leach out the VCM. Nobody knew VCM was bad news then, but the liquor people ditched the idea to be on the safe side.

VCM hair lacquer is now a thing of the past, but the pop bottling and food packaging business is still booming, and the CIA's men recently pointed out that this was because the amount of VCM absorbed from PVC packaging in the diet of your average man was in order of 0.001ppm, and that at this meagre rate of absorption your average man would need to be around for 50,000 years if he wanted to absorb an ounce of VCM. What's more, they remarked, there was no evidence that absorption through the gut rather than through the lungs was in any way harmful. They did not give any indication of what your heavy pop-swagger might expect by way of a daily dosage, or indeed any indication of what dose might be considered lethal, and what safe.

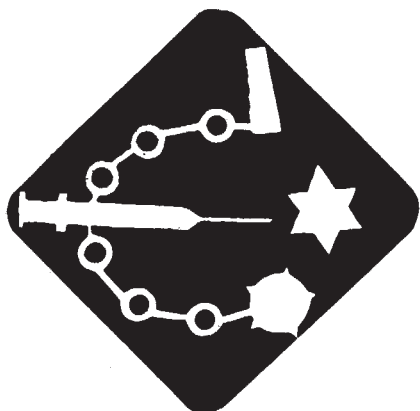
On this last question—the point at which it is safe to absorb a noxious substance and the degree to which an

essential industry can divert its energies to eliminating that substance from the air which its workforce breathes—the CIA has struck a philosophical note. Quite correctly, they point out that PVC is not a luxury product, but a major industrial material which is an integral part of modern life. The car industry, coal mining, domestic and industrial building, water distribution, gramophone record production and various other blessings of civilisation depend upon a ready supply of PVC.

If society demands an absolute zero risk for the 2,000-odd men working on PVC production, then this could be met only by ensuring absolute zero exposures, and since this is not a practical proposition, then the producers would have to stop producing, and life as we know and love it would grind to a halt. With improvements in valves and pipe joints carrying the pressurised gas concentrations as low as 10ppm may be achieved in existing plants, and in entirely new plants, using the most modern technology, 5ppm might be achieved. This, says the CIA, is probably safe, and in any case achievements beyond this point are not conceivable, and so it's up to society to say its piece. If perfection is demanded, the wheels of industry stop turning; if something a little less will do while research proceeds, then we can have our drain pipes and cable covering. And waxing ever so slightly metaphysical, the chairman of the CIA's VCM Committee, Dr A. W. Barnes even volunteered the opinion that five parts per million was arguably closer to nothing than to something, which is stretching public relations to breaking point, even if you are speaking for the combined interests of ICI, BP, Vinatex and British Industrial Plastics.

Red badge of protest

from our Soviet Correspondent



ON the occasion of the great international celebration—that never was of the 250th anniversary of the Soviet Academy of Sciences (or, more correctly, of the old Russian Imperial Academy which it replaced), a small, but significant, "celebration" of the event was held in London by the Medical and Scientific Committee for Soviet Jewry.

The symbol of the meeting, distributed to the participants in the form of a lapel badge, was a redrawing of the official emblem of the Soviet Union—in which the sickle has become a ball and chain, and the hammer the new, scientific and sophisticated weapon of restraining dissidents, the hypodermic. This theme of the repression of the scientific and intellectual community, especially of those members of it who wish to emigrate from the Soviet Union, formed the basis of

the address by Heinz Woolf, who compared the current Soviet policy to that of Ivan the Terrible, who, after St Basil's Cathedral had been completed, had the architect blinded so that no else might commission a similar masterpiece. The current Soviet measures, dismissal and harassment of those scientists who wish to emigrate to Israel, merely continues the same tradition of blocking a brain drain.

A lively discussion followed, on the role which scientific societies and individual scientists outside the Soviet Union can and should play in countering this policy, and the sensitivity of the Soviet authorities to criticism from bodies like the "Royal" Societies and Colleges or publications like *The Times* and *Nature* which they feel to have a certain official or quasi-official status, was particularly stressed as a potential means of counter-attack.

news and views

Recurrent Mach

A STUDENT of the science of science might find it entertaining to try to discover what it is that reawakens interest in Mach's principle every three or four years, why each ingenious suggestion on the subject has almost no effect upon any subsequent ingenious suggestion—except possibly to stimulate the attempt to be more ingenious—and above all why nobody ever actually states Mach's principle. All of which points seem to be instructively illustrated by the article "Relative-distance Machian Theories" by Barbour in this issue of *Nature*. It does seem to be a few years since at any rate *Nature* carried a contribution on the subject: Barbour's suggestion is ingenious and it does not make use of any previous work; he starts from an assertion of something that "Mach's principle, in essence, requires", and he proceeds to treat this requirement itself as a "postulate". But, once again, no statement of Mach's principle! And there never can be a statement of Mach's principle until somebody discloses that Mach actually stated a principle, and in fact nobody has done this. Mach discussed this problem of inertia in a general way, and he seems to have had some idea that inertia depends upon matter in the Universe in the large. But anybody who wants to go further does have to state his own "postulate" because Mach does not help him.

Inertia in the technical sense is a notion of Newtonian physics. Before Newton, the systems of Ptolemy, Copernicus and Kepler each treated a whole Universe—in a very simplified way, of course. Wonderful as they were, they were arid when it came to solving any new problem. Newton came along and said, in effect, that we can break the Universe up—divide and conquer; we may take any small part of the Universe and assert that a frame of reference

exists such that the part obeys laws of motion which take certain forms (Newton's laws as usually stated) when this frame is used. The frame of reference—any inertial frame will serve—is the rest of the Universe in the model. Incidentally, it can be seen at once why the Newtonian treatment cannot apply strictly to the whole Universe, because the treatment depends upon this partition of the Universe. Then Mach came along and, so to say working from the opposite end, redetected what Newton had done. From one point of view, it was no great achievement to suggest that an inertial frame might have something to do with the Universe in the large, when all the time the inertial frame is the Universe in the large. From another point of view, Mach did something great in making people wonder why the Newtonian treatment is so successful. People are still wondering about this, and Barbour's paper is an obviously interesting endeavour to push it back to more rudimentary postulates. It might be inappropriate here to discuss details, since he remarks that he is preparing a paper on a different model with more interesting properties.

There is still the general question as to whether such ideas carry over into relativistic models. Barbour evidently believes that his treatment will extend to special relativity; this would be natural, for special relativity also is founded upon the use of inertial frames. It is outside his present scope to say much about general relativity. In general relativity, however, every problem produces an entire universe (in general, not an interesting one); so there is no Newtonian partition and therefore Mach's problem does not arise in the same way. Some writers have indeed, produced sophisticated criteria as to whether any particular general relativity model is "Machian" or not, but this seems to be somewhat far from Barbour's interpretation of "Machian".

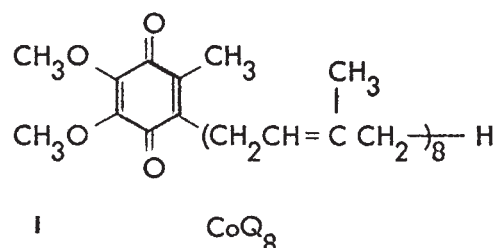
W. H. MCCREA

Possible new antimalarial drugs

ALTHOUGH it is well established that malaria parasites inside host red blood cells utilise glucose, the extent to which this process provides the parasites with energy has been little explored. One of the technical problems hindering such an investigation was the difficulty of distinguishing between the biochemical processes of the intracellular parasite and those of the host cell. Until recently much effort was directed towards separating the parasite from host erythrocytes and from other cellular blood elements, particularly white cells and platelets, the activities of which could invalidate biochemical assays of, for example, oxygen consumption and the action of classical metabolic inhibitors. Homewood and her colleagues (*Proc. helminth. Soc. Wash.*, **39** (special issue), 382; 1972), have utilised a technique based on the observation that the pigment of erythrocytic stages of the rodent malaria parasite *Plasmodium berghei*, clumps when exposed to a low concentration of chloroquine, and that this process is dependent on energy (Warhurst and Robinson, *Trans. R. Soc. trop. Med. Hyg.*, **65**, 11; 1971). From a study of the action of various respiratory inhibitors on pigment clumping induced by chloroquine, Homewood and her colleagues postulated that these parasites possess a very unusual, branched electron transport chain. The major branch, they suggested, terminates in an unidentified acceptor of parasite origin, and they believe that the minor branch passes electrons finally to

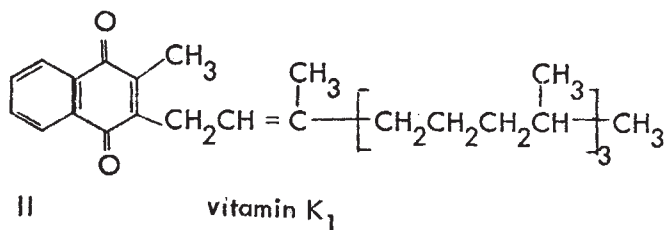
oxygen by way of a cyanide-sensitive cytochrome oxidase system.

There is now good evidence to show that the erythrocytic stages of a variety of *Plasmodium* species, including *P. berghei*, do indeed contain cytochrome oxidase (mainly in the mitochondria), and that the enzyme occurs also in the mosquito and pre-erythrocytic liver stages (Howells *et al.*, in *Comparative Biochemistry of Parasites*, Academic, London and New York, 1972). The idea that electron transport mechanisms may play a vital part in the malaria parasite has now been exploited by Wan, Porter and Folkers (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 952; 1974) who have synthesised a series of structural analogues of coenzyme Q₈ (I).



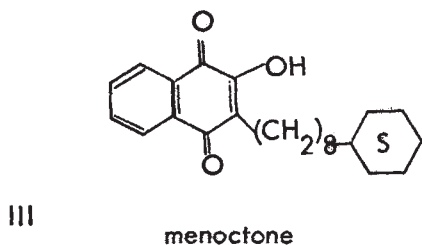
During the Second World War antimalarial drug screening

programme Fieser and his associates (*J. Am. chem. Soc.*, **70**, 3151; 1948) synthesised several 2-hydroxy,1,4-naphthoquinones that proved to be highly inhibitory to growth of the duck malaria parasite *P. lophurae* and two of these were eventually tested in man. They produced, however, some side reactions—apparently due to an effect on blood clotting mechanisms—that were, fortunately, antagonised by the administration of vitamin K1 (II). Subsequent investigations

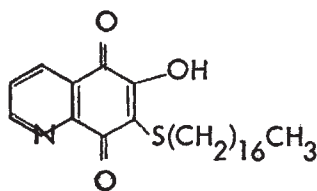


showed that these antimalarials inhibit mammalian and parasite respiration, an effect that could be reversed in the former by the addition, *in vitro*, not of vitamin K1 but of ubiquinone 10 (also referred to as coenzyme Q₁₀, or CoQ₁₀).

It is now known that *P. lophurae* synthesises ubiquinone 8 and 9 (Rietz *et al.*, *Int. Z. VitamForsch.*, **37**, 405; 1967) and that ubiquinone 7 and 8 are synthesised by *P. berghei* and two simian malaria species which do not contain vitamin K₁ (Skelton *et al.*, *J. med. Chem.*, **13**, 602; 1970). These observations have opened a new line of thought as to the mode of action of the naphthoquinones, as well as providing a unique target for selective toxicity, since the host tissues of both birds and mammals synthesise a different coenzyme, namely ubiquinone 10. It was soon demonstrated that both the older 2-hydroxy, 1,4-naphthoquinones as well as some newer members of the series, including menoctone (III), inhibit DPNH



oxidase and succinoxidase systems *in vitro*, both systems being ones in which CoQ plays an essential part. These observations encouraged the synthesis of further compounds structurally related to ubiquinone 8, the most recent of which are 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones (Wan *et al.*, *loc. cit.*). One of them, the heptadecyl derivative (IV), combines a high level of activity both against the blood stages of *P. berghei* and especially against the tissue stages of the avian parasite *P. gallinaceum* with a very low level of toxicity to the host. There are thus two chemical series, one characterised by menoctone (III) with its 8 carbon alkyl,



3 cyclohexyl sidechain, the other by compound IV with 16+1 carbons in the saturated straight alkyl chain, both of which show a high degree of antimalarial activity and low host toxicity. It seems quite possible that they function as antimetabolites by blocking one or more sites concerned with the biosynthesis of CoQ₈ within the malaria parasite. In the menoctone series activity against *P. berghei* is lost as the chain length is either decreased or increased, both in relation to the blood (Berberian and Slighter, *J. Parasit.*, **54**, 999; 1968) and tissue stages (Berberian *et al.*, *ibid.*, 1181).

Menoctone has been shown to cause selective structural damage to the well developed mitochondria of the tissue stages of avian malaria (Beaudoin *et al.*, *Milit. Med.*, **134**, 979; 1969). Although in the blood stages of *P. berghei* the mitochondria appear to be relatively primitive, it now seems clear that they do play an important part in the production of energy through the electron transport chain. It is therefore interesting to note that menoctone also induces ultrastructural changes in these mitochondria (Howells *et al.*, *Ann. trop. Med. Parasit.*, **64**, 203; 1970).

These compounds thus approximate to the definition of the ideal antimalarial which has been stated to be one that would (among other virtues) combine action against all stages of the parasites with minimal toxicity to the host. Although preliminary studies with menoctone against *P. falciparum* in man (*Tech. Rep. Ser. Wld Hlth Org.*, No. 529, 70; 1973) and the 7-octadecylmercapto analogue of IV against *P. cynomolgi* in monkeys (Wan *et al.*, *loc. cit.*) have been disappointing, the basic experimental data and theoretical considerations indicate that the further exploration of these potential antagonists of CoQ₈ may well provide a rare opportunity for the rational synthesis of an antiparasitic agent with highly selective toxicity, the dream of every experimental chemotherapist since Paul Ehrlich. A word of caution, however, is necessary. The observations of Howells *et al.* (*loc. cit.*; 1970) indicate, perhaps not surprisingly, that the malaria parasite may be equipped already with a means of overcoming such interference with its vital respiratory processes.

W. PETERS

Another bursal equivalent

STUDY of the lymphoid system in birds has revealed a thymus at the anterior and bursa (of Fabricius) at the posterior end. Both organs are thought to play a primary part in the development of the lymphoid system and immunological responsiveness. Stated in its simplest form the notion is that the thymus produces T cells which are responsible for cell-mediated immunity (that is against foreign grafts) and the bursa emits B cells which produce humoral antibody.

Initially it seemed that these two modes of immunity, like the antithetic East and West of Rudyard Kipling, should never meet. In mammals, particularly mice, the thymus is present and it produces T cells and these cells are responsible (at least in part) for cell-mediated immunity. The bursa in mice is less obvious and murine T cells collaborate with murine B cells (B stands for bursal equivalent here) to facilitate the production of antibody by the B cells. All this looks sensible and seems to indicate that birds and mammals differ in their lymphoid systems and manner of immunological responsiveness. (It would be more accurate to say that chickens and mice differ.) But immunologists are spending much time and energy in trying to reconcile these apparent differences, and the definition of a mammalian bursal equivalent in particular has become a sort of crusade. Owen, Cooper and Raff raise the banner once again on page 361 of this issue of *Nature*. Owen *et al.* first assem-

bled a series of fluorescein-conjugated, class specific, anti-mouse immunoglobulin antibodies in order that they could identify B lymphocytes by direct absorption of the fluorescent antibody and subsequent counting of stained cells in an appropriate microscope. They make the assumption that surface immunoglobulin on a morphologically identifiable lymphocyte is sufficient definition of the cell as a B cell.

These methods were then applied to liver and spleen of foetal mice of various gestational ages. It was found that until 17 days of foetal life, no B (or T) lymphocytes were detectable. They then took 14-day foetal liver and cultured it *in vitro* for up to 7 days. Within 3 days 0.1% of all cells were stained and within 7 days as many as 3%. Over the same time course there was found in the explanted livers an increase in the proportion of small lymphocytes from 0% to 3%. In these circumstances Owen *et al.* very reasonably conclude that B lymphocytes develop in relatively large numbers in foetal liver in tissue culture. They also point out that, whether or not their deduction that the foetal liver is a bursal equivalent proves to be validated, the system of experimentation they have adopted provides an opportunity for the study of the putative class switch in immunoglobulin synthesis, and the generation of antibody diversity in the absence of T cells.

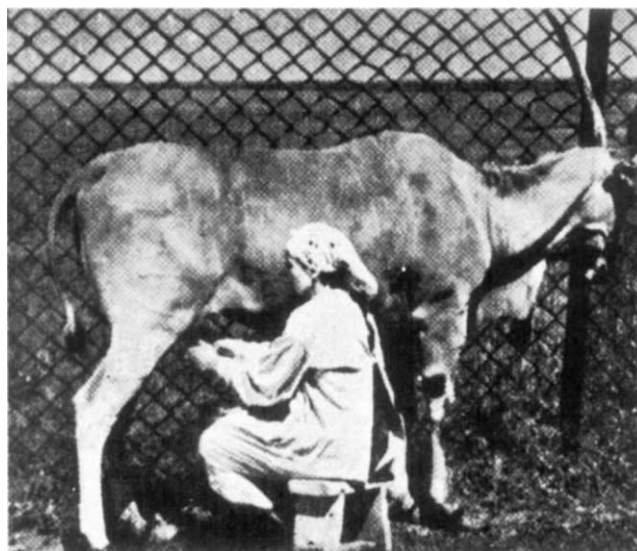
It is being found with increasing frequency that non-lymphoid cells can demonstrate some of the best recognitional characteristics of T and B lymphocytes (see, for example, Elliott, Kerbel and Phillips, *Nature*, **248**, 514; 1974). It is to be hoped that this does not prove true of surface-bound immunoglobulin, because if it did Owen *et al.* would have to show that their fluorescent cells were morphologically small lymphocytes.

A. J. S. DAVIES

Protein for population

from our *Animal Ecology Correspondent*

OF the many problems facing policy makers of sub-Saharan Africa today, the increasing protein deficiency for the spiralling human population is about the greatest. It is of historical interest to note that protein production has traditionally rested on the performance of domestic livestock. In some African countries the problem has been tackled by the importation of protein-rich foods. But the high price of these foods renders them unavailable to all but 25% of the population. The other 75% rely on caterpillars, maggots, snails, fish and wildlife for their protein.



African eland being milked at Askaniya Nova in the Crimea, USSR, where there is a domesticated herd. The milk has more fat and protein and less water than that of domestic cattle and is more resistant to some bacteria (from Crawford, *Oryx*, **12**, 321; 1974).

Asibey has pointed out (*Biol. Cons.*, **6**, 32; 1974) that natural meat, or bushmeat as it is called, represents big money. In one market in Accra species such as hare, giant rat, grasscutter, duiker, green monkey and bushbuck fetched US \$0.46 per pound. During 18 months the cash turnover from 348,000 pounds (158,000 kg) of meat was US \$160,000. If planners need to see the future in terms of money they should take a look at their own back garden. This, above all, may convince them that the true solution rests with the effective and efficient exploitation and management of naturally occurring wildlife.

There are other, more important, reasons why the bushmeat industry should be encouraged and given the protection of careful management. These concern conversion and growth efficiencies of wild game compared with domestic stock. Eland show a liveweight gain of 0.73 pounds (0.33 kg) per day; domestic cattle on reasonably managed rangeland in East Africa gain about 0.3 pounds (0.14 kg) per day. Crawford (*Oryx*, **12**, 351; 1974) uses this example to highlight the need for field studies. When eland and cattle are kept in the same laboratory their conversion efficiencies are similar. But on the arid plains the eland's performance is characterised by lability. By allowing its body temperature to rise with the ambient temperature—and it can even exceed the air temperatures and radiate heat—it has no requirement to sweat or pant. The saving of body water, critical in many parts of Africa, is considerable. Of course it is only able to allow its body temperature to rise because of certain adaptations to its heat balance system. These include a *reta mirabilia* overlying the brain which functions as a heat sink. Furthermore, bushmeat species are remarkably resistant to infections that kill domestic stock, and they seldom destroy the

phytosociological structure of their habitats.

Both Asibey and Crawford independently make a plea for rational and scientific management of natural stocks. Clearly, if the resource is to withstand fairly heavy exploitation then close scrutiny must be made of population processes in the wild. Crawford sees two possible ways in which management could be directed. One is towards the establishment of monocultures of eland, wildebeest, gazelle or kob. The other is towards polyculture establishment using a range of game animals occupying different feeding niches and maintaining a great complexity and diversity of vegetation. Ecologically speaking the polyculture system is the more viable since overgrazing and overbrowsing are less likely to occur than in monoculture.

With the standing crop of bushmeat being around 70,000–100,000 pounds per square mile (12,000–17,400 kg km⁻²) and of domestic stock around 11,000–16,000 pounds per square mile (1,900–2,800 kg km⁻²), it is inconceivable that this valuable resource will have to wait long before heavy exploitation begins. And neither it should, if the people of Africa are to have meat to eat. Asibey's plea for intensive and detailed management studies must not go unheeded by the architects of the new Africa.



African buffalo. (Photo.: M. Crawford). Their long gestation period (compared with domestic cattle) ensures that calves are born in the rainy season.

Galapagos seismic gap filled

from Peter J. Smith
Geomagnetism Correspondent

ALTHOUGH all active oceanic ridges are marked by chains of shallow focus earthquakes, the density of epicentres is far from uniform throughout the ridge system. There are, for example, far fewer events along fast-spreading ridges than along slow-spreading ridges. Macdonald and Mudie (*Geophys. J.*, **36**, 245; 1974) have examined earthquakes of magnitude 5.0+ along spreading ridge sections (that is, excluding those events associated with known transform faults), as recorded during the years 1961-1969 by the World Wide Standardised Seismograph Network (WWSSN), and find that on average there were 8 shocks per 1,000 km of ridge for the northern mid-Atlantic Ridge, where the spreading rate is 12 mm yr⁻¹, but less than one shock per 1,000 km along the East Pacific Rise, where the spreading rate is 35-50 mm yr⁻¹. Moreover, fast-spreading ridges are particularly prone to apparent gaps in seismic activity of up to hundreds of kilometres in length. Along the East Pacific Rise between 36° S and 49° S, for example, there are seismic gaps (as revealed by WWSSN records) as long as 1,400 km.

Another area in which there seems to be a seismic hiatus, according to the WWSSN, is the 600-km long central Galapagos spreading centre, a ridge section spreading at a half-rate of 32.5 mm yr⁻¹ between the Cocos plate to the north and the Nazca plate to the south. But is earthquake activity truly absent from this region, or is the gap in seismicity merely a reflection of the low sensitivity of land based stations? To find out, Macdonald and Mudie used sonobuoys in an attempt to detect seismicity at close range along the Galapagos spreading centre—in other words, to see if, notwithstanding the lack of large earthquakes, they could detect microearthquake (magnitude less than 4.0) or earthquake swarm activity. The attempt succeeded. During 45 h of recording time (not continuous) Macdonald and Mudie detected an average of 15 events an hour near the ridge axis and up to 80 shocks an hour at the peak of a swarm which occurred a few kilometres from the ridge. Magnitudes ranged from $m_b = -0.4$ to $m_b = +0.8$. This is the first time that the microseismicity of a fast-spreading ridge with no activity detectable by the WWSSN has been measured.

The association with an active ridge leaves little doubt that the recorded microearthquake activity is caused by seafloor spreading processes, although

the specific origin is more difficult to determine. Sykes (*J. geophys. Res.*, **75**, 6598; 1970), who investigated ridge swarms elsewhere recorded by the WWSSN, attributed such swarms to volcanic, hydrothermal or magmatic processes on the ridge crest; and it seems probable that one or more of these processes are also responsible for the Galapagos events. Williams *et al.* (*Eos*, **54**, 244; 1973) suggested that hydrothermal circulation may be an important method of heat transfer at the Galapagos ridge crest, for heat flow there can be greater than 30 heat flux units and narrow, near-bottom, positive water temperature anomalies of several hundredths of a degree have been found over the crest near, as it now turns out, the source of the micro-earthquake activity. But what is probably the most convincing evidence comes from the Macdonald-Mudie study itself. The b value for the Galapagos events (the frequency-magnitude parameter in the relation $\log N = a - bm$, where N is the number of events of magnitude m , or greater) is very high at 3.0, which is consistent with a volcanic or magmatic source but not with the generally much lower b values for fracture zone events.

Flowing superfluid films

from Peter V. E. McClintock
Condensed Matter Correspondent

THE thickness of a superfluid helium film below 1 K is reduced by an increment which varies quadratically with the velocity at which the film is flowing, according to an experiment by Williams and Packard of the University of California at Berkeley (*Phys. Rev. Lett.*, **32**, 587; 1974).

Some of the most dramatic demonstrations of superfluidity in liquid ⁴He below its superfluid transition temperature at 2.18 K are found in the remarkable phenomena associated with helium films. Any surface dipping into the liquid rapidly becomes covered with a film whose thickness depends on the nature of the surface, and the height above the bulk liquid, but which is usually a few hundred Å.

Up to a certain critical velocity the film encounters no resistance to its motion and, being thus enabled to flow freely at several tens of cm s⁻¹, can act as a channel through which relatively large volumes of the liquid can be transferred: in fact the film can act as a syphon through which a small vessel of helium will empty itself in a matter of minutes, the helium flowing up the inner wall and then down the outside.

The film is held in position by the

attractive Van der Waals force between the wall and the helium atoms, and measurements of the thickness of static films are found to be in reasonable agreement with theoretical predictions based on the assumption that the film always adjusts itself so as to minimise its total potential energy in the combined Van der Waals and gravitational potentials.

In the case of a flowing film, however, it may not be possible for the thickness to reach this equilibrium value since the velocity of flow is limited to the critical velocity, above which dissipative processes set in very rapidly. Although the situation is very complicated, Kontorovich was able to show (*Zh. eksp. teor. Fiz.*, **30**, 805; 1956) that, under these conditions, the thickness should be reduced by an amount depending on the square of the flow velocity.

Subsequent experiments above 1 K in a number of laboratories, however, demonstrated conclusively that the thickness was actually independent of the velocity. A possible explanation of this apparent conflict between theory and experiment was given by de Bruyn Ouboter (*J. low Temp. Phys.*, **12**, 3; 1973) who pointed out that Kontorovich's analysis had considered equilibrium conditions only within the film itself and had ignored the influence of the surrounding helium vapour: a flowing film in internal dynamic equilibrium would clearly be in disequilibrium with the vapour which, by condensing, would tend to bring the thickness back to its value for a static film. One obvious experiment to test this hypothesis was to repeat the measurements at temperatures below 1 K where the saturated vapour pressure is so small that condensation on the moving film would be a negligible effect. This is the experiment which has now been carried out by Williams and Packard.

Their experimental cell was cooled by means of a dilution refrigerator to temperatures well below 1 K, and was designed to measure the transfer rate of helium between two reservoirs, while at the same time monitoring the thickness of the flowing film. The latter measurement was performed by making the film flow between a pair of capacitor plates so that a change in its thickness resulted in a small change in their capacitance, which could be measured externally. By this means, changes as small as 2 Å could be detected. By using a second pair of capacitor plates to form the walls of one of the reservoirs, the volume of liquid present could be measured as a function of time, thus enabling the film flow rate to be deduced. The authors found that the film became considerably thinner when flowing, than when it was static. At 0.26 K with a

critical velocity of 28 cm s^{-1} the film thickness was reduced by $56 \text{ \AA}$.

By investigating transient effects which occurred as the flow started and stopped, they were able to measure the film thickness for velocities less than the critical velocity and they found that the thinning was proportional to the square of the velocity as had been predicted by Kontorovich.

These measurements provide a satisfying demonstration of the general validity of Kontorovich's analysis. They also, however, demonstrate the corrections of de Bruyn Ouboter's suggestion of the importance of vapour condensation for films at temperatures above 1 K , which has implications for a whole range of other experiments and proposed experiments. Williams and Packard point out, in particular, that by providing a dissipative mechanism, such condensation processes may be responsible for the experimental non-appearance of persistent currents in helium films above 1 K . So that this latter hypothesis may be put to the test, it is to be hoped that further experiments seeking persistent currents in helium films will soon be carried out at temperatures well below 1 K , where the influence of the vapour is negligible.

Complexities of the Afar triple junction

from Ian Gass

DURING the past five years the Afar has been the site of intensive investigations by French, German and Italian research teams, as well as by individual scientists from the United Kingdom and United States. A meeting on the Afar region, held at Bad Bergzabern, West Germany, from April 1-6, was anticipated with mixed feelings. Undoubtedly much new information would be available, but would it in any way make the processes at this unique terrestrial triple junction any more explicable? These misgivings were unfounded: the symposium was an outstanding success. The lasting impression is of the excellence and abundance of the German geophysical data and of the French and Italian field, structural and petrochemical studies.

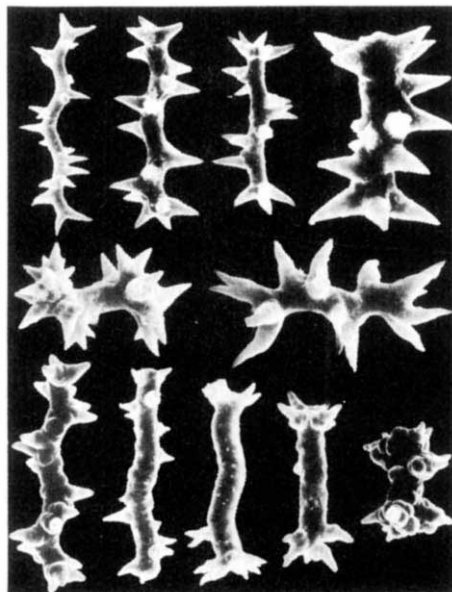
The following points were repeatedly emphasised; that (1) the continental crust beneath Afar has been markedly attenuated; (2) the underlying upper mantle is anomalous to depths of 300 km with maximum recorded P wave velocities in the range $7.3\text{--}7.4 \text{ km s}^{-1}$; (3) movement, both vertical and horizontal, volcanic activity and deformation are episodic with periods of activity interspersed with intervals of quiescence; (4) the major volcanic activity

commenced some 34 Myr ago and that older dates on trap basalts are open to considerable doubt; (5) the disposition of volcanic rock types is far more complex than originally thought; (6) the precise demarcation line between the Nubian, Somali and Arabian plates can be identified more closely than before within the Afar depression but that its position on or around the Danakil Alps is still a matter of personal prejudice.

Both R. Black (University of Montpellier) and W. H. Morton (University of Addis Ababa) convincingly demonstrated that crustal attenuation had taken place by a process of rotating fault blocks. Morton proposed that the angle of faulting was a direct reflection of the degree of attenuation in the upper brittle crust while the lower crust deformed plastically. The German seismic data, reported by H. Berckhemer (University of Frankfurt), and the gravity information (J. Makris *et al.*, University of Hamburg) proposed anomalous mantle under Afar though that below the highland is normal. The gravity model presented showed a mantle of 3.15 g cm^{-3} within 20 km of the surface and thinnest beneath the rift zones; a space form similar to that proposed by Gass in 1969 on petrological criteria is indicated. Magnetotelluric measurements (A. Berkbold, University of Munich), deep electrical conductivity (H. Porath *et al.*, University of Dallas) and surface wave dispersion (R. C. Searle, National Institute of Oceanographic Sciences, Wormley) all support the presence of low density mantle at high level beneath Afar. Farther to the south a similar space form was proposed for the Kenya rift by R. E. Long (University of Durham), J. D. Fairhead (University of Leeds) and N. Logatchev (Institute of the Earth's Crust, Irkutsk) on teleseismic, gravity and petrological data respectively, although the depth to anomalous mantle varies somewhat.

Time and time again speakers emphasised the periodicity of activity. H. Faure (Centre National de la Recherche Scientifique, Bellevue) demonstrated spasmodic vertical uplift in the order of 0.3 mm yr^{-1} around the Gulf of Tadjura. F. Berberi *et al.* (University of Pisa) and J. Varet (University of Paris) also emphasised the spasmodic nature of uplift which they dated as occurring mainly between $45\text{--}40$, $25\text{--}20$ and $5\text{--}2 \text{ Myr}$ with volcanic maxima between. Although the generally spasmodic nature of events was acknowledged, the longest pause—that proposed by R. W. Girdler and P. Styles (University of Newcastle-upon-Tyne) for a cessation of spreading in the Red Sea between $34\text{--}5 \text{ Myr}$ —was questioned. Many found it difficult to accept that all was quiet in the Red Sea while vol-

Inside a sponge



To anybody whose familiarity with sponges goes no further than the bathroom, it may come as a surprise that the bath sponge *Euspongia* is just one of many examples of the sponge phylum Porifera. The strange-looking structures in the figure shown here make up the skeleton of another form of sponge, *Cliona dioryssa*, which burrows into calcareous rocks. This species is one of eight burrowing sponges which Dr Klaus Rützler has been studying in Bermuda (Smithson. Contrib. Zool., No. 165; 1974). Although *Cliona* is sufficiently similar to *Euspongia* for both to be included in the same broad class, the Desmospongia, the skeletons are different; *Cliona*'s skeleton is made up of the siliceous spicules shown here whereas that of *Euspongia* is composed of a horny substance called spongin.

canism, uplift, crustal attenuation and deformation were going on all around.

Petrologically the picture has altered from the simplistic views of the late 1960s and this is mainly due to the recognition of tholeiitic basalts within the Ethiopian Trap series as well as within the depression. Although generations of basaltic liquids within an area of high heat flow (the use of the term mantle plume being deliberately avoided) is generally accepted, it is quite apparent that factors other than depth of equilibration are relevant and more correlated field, age, petrographic and geochemical data are required before a realistic petrogenetic model can be erected.

Lastly, and perhaps appropriately, the position of the dividing line between the three plates, although becoming more precisely defined, is still masked by the

complexity of surface deformation and the super-abundance of Recent volcanic products. Over large areas it is, within limits, a matter of choice as to where it is put—and that alone will keep the arguments going.

Support for deep mantle convection

from Peter J. Smith

Geomagnetism Correspondent

ASSUMING that the engine driving the Earth's lithospheric plates involves some sort of convection in the mantle, do the convection cells reach down to the core-mantle boundary or are they relatively shallow? Views on this point have changed significantly since the idea of motions in the mantle was first proposed. Put briefly and simply, convection as originally envisaged was regarded as mantle wide, with the currents moving the passive lithosphere by viscous coupling. As the new global tectonics developed during the 1960s, however, the nature of the asthenosphere became clearer and the lithospheric plates themselves came to be seen as possible driving forces (acting under gravity, for example). The result was that convection cells became conceptually shallower, being limited to the upper mantle or even to the lithosphere-asthenosphere. More recently, sporadic attempts have been made to reverse this trend by reviving mantle-wide convection but have generally received little support. The latest such attempt is by Robinson (*Earth planet. Sci. Lett.*, **21**, 190; 1974) who has managed to refute the two serious criticisms of mantle-wide convection put forward some years ago by Ringwood (*ibid.*, **14**, 233; 1972).

Robinson's two-dimensional model involves steady cellular convection driven by heat from below. The cells are 3,000 km deep (the thickness of the mantle) and 1,400 km wide (which is within observed trench-ridge separations), but their most important characteristic is that their limbs are narrow. In other words, the convection of heat between the core-mantle boundary and the Earth's surface layer takes place within a narrow (100–200 km) boundary layer, the vertical segments of which form narrow ascending and descending 'plumes'. This immediately avoids Ringwood's first problem, which is that the necessary 200° C mean temperature difference between the ascending and descending currents in the conventional wide-limbed mantle-wide convection model would imply gravity anomalies at the Earth's surface more than 100 times greater than those observed. In Robinson's model the corresponding temperature difference is of the order of 1,000° C but is con-

fined to the narrow plumes. The resulting gravity anomalies are thus comparable to those actually observed.

The second problem that Ringwood pointed to was that of "re-establishing a superadiabatic gradient by thermal conduction after completion of half a turn of the cycle", the point being that superadiabatic gradients are necessary to drive the convection currents. According to Ringwood, the time required for conduction through 3,000 km of mantle would be 2.5×10^{10} yr, so that "only one convective overturn could have occurred throughout the history of the Earth". But according to Robinson, this problem does not arise in his model because the time scale for conduction through a narrow boundary layer is much shorter than that of the convective cycle. In any case, the superadiabatic gradient in the plumes (the temperature gradient inside the cells is adiabatic) could be maintained by energy stored in the core. Ringwood rejected this notion on the grounds that unacceptable radioactive abundances would be required in the core, and that even if the necessary deep heat could be found it would lead to unacceptably high surface heat flow. Robinson argues, however, that the narrow plumes of his model avoid the need for high surface heat flow and that one can envisage heat sources in the core which do not involve radioactivity.

Advances in lichenology

from a Correspondent

PROGRESS and problems in lichenology were considered at a meeting at the University of Bristol on April 8–10. It was the first international symposium held in Britain devoted solely to lichens and was organised jointly by the Systematics Association and the British Lichen Society.

Some fundamental questions as to the nature of lichens were raised by P. W. James (British Museum (Natural History)) and A. Henssen (Botanical Institute, Marburg) who reported convincing evidence that a single fungal partner with different types of algae (green and blue green) can produce morphologically very different individuals traditionally placed in different genera. The production of some chemical components used in lichen taxonomy may also be affected by the algae present. The use of chemical characters in lichen taxonomy was reviewed by D. L. Hawksworth (Commonwealth Mycological Institute, Kew) who suggested guidelines for the taxonomic treatment of chemical races in lichens, and R. W. A. Oliver (University of Salford) demonstrated the value of mass spectrometry and high pressure

liquid chromatography for the accurate determination of substances in small amounts of herbarium specimens. Lichen taxonomy is also being clarified by studies of ontogeny (Henssen), algal components (E. Tschermak-Weess, Botanical Institute, Vienna) and by scanning electron microscopy (M. E. Hale, Smithsonian Institution).

On the ecological side F. Rose (King's College, London) showed the value of lichens as indicators of ancient woodlands in relatively unpolluted parts of Britain. M. R. D. Seaward (Trinity and All Saints' Colleges, Leeds) reported on the performance of *Lecanora muralis* in urban areas and A. Fletcher (University College of North Wales, Anglesey) described the ecophysiology of marine and maritime lichens. Distributional studies on a world scale (P. W. James) and in Britain (B. J. Coppins, Royal Botanic Garden, Edinburgh) were also reported. A novel technique for measuring lichen growth rates was proposed by R. A. Armstrong (University of Oxford) and R. H. Bailey (Department of Extra-Mural Studies, London) pointed to gaps in knowledge of the establishment and dispersal of lichens.

Physiological studies formed the basis of several contributions. Sulphur uptake and metabolism using radioactively-labelled sulphur — subjects relevant to the use of lichens as air pollution indicators — were reported by B. W. Ferry (Bedford College, London) and the uptake of heavy metals in lichens was discussed by D. H. Brown (University of Bristol). Nitrogen fixation and transfer in lichens continue to provide an interesting field for research as was shown by J. W. Millbank (Imperial College, London). The physiology of symbiosis itself and the transfer of carbohydrates were discussed by J. F. Farrar (Imperial College Field Station, Silwood Park) and D. J. Hill (University of Newcastle upon Tyne).

D. C. Smith (University of Oxford) compared the lichen symbiosis with other symbiotic systems; he pointed to many similarities and stressed that from the laboratory standpoint lichens were ideal organisms in which to study symbiosis itself. The concept of controlled parasitism of the algal partner by the fungus may, however, be preferable to one of symbiosis in lichens.

In conclusion R. Santesson (University of Stockholm) pointed to the resurgence of interest in lichens and the stimulus which this meeting provided for further work. It is clear that lichenology is a field in which many problems, both old and new, require further research, but also one in which it is still relatively easy to make important original contributions.

Sugar receptor sites in the fly's tongue

from our
Insect Physiology Correspondent

SINCE the isolation of a 'sweet-sensitive protein' from the bovine tongue there has been increasing interest in the properties of sensory receptor substances and receptor sites. Von Frisch studying the honeybee in 1935 and Dethier studying the blowfly in 1955 failed to discover configurations that were common to the stimulating carbohydrates and were absent in the tasteless ones. But their results underlined the extreme specificity of insect receptors as compared with those of mammals. Dethier observed, however, that the weakly stimulating sugar, mannose, markedly reduced stimulation by fructose but had no effect on glucose sensitivity. Evans (1961) inferred that there must be at least two different sites in the sugar receptor of the blowfly tongue, a 'glucose site' and a 'fructose site'.

Shimada *et al.* (*J. Insect Physiol.*, **20**, 605; 1974) have now re-investigated this problem in the large labellar hairs of the flesh fly *Boettcherisca peregrina*. In earlier work these authors had found that the sugar receptor in this fly with respect to sucrose could be modified by treatment with the sulphhydryl reagent, *p*-chloromercuribenzoate (PCMB). PCMB seemed to react with sulphhydryl groups in proteins of the sugar receptor, but not at the sugar-binding site; it apparently blocked a change in the permeability of the receptor membrane. Shimada *et al.* have now made a detailed study of the effects of PCMB on the electrophysiological response of single receptor hairs, to fructose, glucose and other sugars.

It turned out that there is a striking difference in the effect on the response to glucose and fructose: response to glucose was completely repressed; res-

ponse to fructose was unaffected (see figure). This constitutes the first direct demonstration of the existence of a 'glucose site' and a 'fructose site' as suggested since Dethier (1955). When a range of other monosaccharides was tested it was found that these fell into two groups: D-fructose, D-fucose, and D-galactose react chiefly with the 'fructose site'; D-arabinose, L-fucose, L-arabinose, L-sorbose, D-xylose, L-glucose and D-glucose mostly with the 'glucose site'. In the disaccharides sucrose and maltose the glucose moiety predominates and both react with the 'glucose site'. High concentrations of glucose exert no specific protective effect against PCMB treatment: this supports the view that PCMB may not react at the glucose-binding site but at a different site necessary for stimulation by D-glucose.

From a discussion of these results and the known equilibrium changes in the conformations of a single sugar in aqueous solution, Shimada *et al.* adopt the more precise terms 'pyranose site' and 'furanose site' (instead of 'glucose site' and 'fructose site') representing the sites which respond especially to sugars in the pyranose and furanose forms respectively. The pyranose group was further divided into two subclasses according to the presence or absence of three successive equatorial hydroxyl groups regardless of their positions.

Positive control in the test tube

from Benjamin Lewin
Molecular Genetics Correspondent

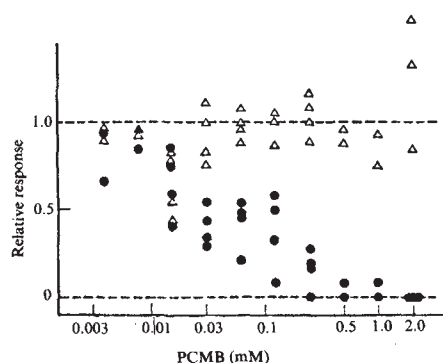
TURNING on gene expression *in vitro* has in general proved to be more difficult than switching it off, for although several systems have been developed in which specific repression takes place in the test tube, characterisation of positive control systems has been less successful. The positive control system investigated in most detail to date is the catabolite activator protein stimulated by cyclic AMP, whose mediation is necessary for expression of several inducible and repressible operons. Although this system seems to act on the initiation of transcription, little is known about the molecular details of its activity. Several papers in the March issue of the *Proceedings of the National Academy of Sciences* now take the analysis of positive control *in vitro* a stage further in three systems.

The first positive control system to be suggested was that of the arabinose operon, in which genetic analysis implied that a repressor protein might be converted by arabinose into an inducer protein (instead of simply suffering the inactivation characteristic of negative

control systems). Lee *et al.* now report (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 634; 1974) the development of a system in which the regulator protein, activated by arabinose, and the catabolite activator protein, stimulated by cyclic AMP, are both needed for initiation of transcription. Using a template of phage λ carrying the arabinose genes, synthesis of *ara* messenger RNA can be followed by hybridisation first with λ DNA (to remove phage sequences) and then with λ *ara* DNA. This system seems completely dependent on the catabolite activator protein, is stimulated by the arabinose regulator protein in the presence of arabinose, and is inhibited by fucose (an anti-inducer of the operon). These results support previous suggestions that both the regulator protein and the catabolite activator protein must join with RNA polymerase to initiate transcription. Further analysis of this system may be important in helping to define how regulator proteins may interact with each other and with DNA.

Phage lambda itself provides a system under both negative control (lambda repressor prevents expression of integrated lysogenic lambda genomes) and positive control (needed both for the establishment of lysogeny and also for lytic development). Establishment of lysogeny depends on two phage functions (*cII* and *cIII*) in addition to the repressor coded by the *cI* gene, and also upon host genes. One of the host functions has now been identified by Belfort and Wulff (*ibid.*, 779), who have isolated *hfl*⁻ mutants of *Escherichia coli* that show very high frequencies of lysogenisation after lambda infection. Because the *hfl*⁻ mutation is recessive, it is likely that it is caused by the loss of an active *hfl*⁺ gene product, which usually prevents establishment of lysogeny. Because λ phage DNA lacking an active *cIII* gene continues to achieve the same high frequency of lysogenisation in *hfl*⁻ cells that is displayed by wild type λ , it is possible that the *hfl*⁺ function is usually antagonised by the *cIII*⁺ gene product.

That the host catabolite activator system also is implicated in establishment of lysogeny is suggested by the reduction in lysogenisation frequency in mutants defective in this system. Belfort and Wulff found that whereas these host mutants display a slight reduction in frequency of lysogenisation by λ ⁺ phages, they show a massive reduction in lysogenisation by *cIII*⁻ mutants. That the catabolite activator and *hfl* systems both act in the same pathway as the *cIII* phage gene product is shown by the observation that the inability of catabolite activator host mutants to lysogenise λ *cIII*⁻ mutants is reversed by the introduction of an *hfl*⁻ mutation. Because catabolite re-



Depression of responses to fructose and to glucose by treatment with *p*-chloromercuribenzoate at graded concentrations. Ordinate: response relative to that before treatment. Duration of treatment with PCMB was always 3 min. Δ , 0.1M fructose; $\bullet$, 0.2M glucose.

pression is normal in *hfl*⁻ strains, it is possible to exclude the idea that this gene specifies a component of the catabolite repression system. The roles of the catabolite activator protein and the *hfl* gene product in establishing lysogeny are not clear, but it is likely that these systems may be more intricate than previously suspected, and further components may remain to be identified.

A different form of positive control, so far demonstrated only with the mRNAs of phages T3 and T7, is exercised by processing systems. The RNA polymerase enzyme of the host, *E. coli*, transcribes one large messenger *in vitro* from the early region of these phages (corresponding to 20% of the DNA, located at its left end). But infected cells contain five, much smaller, early T7 messengers. Recently it has seemed likely that the discrepancy is due to the presence in the cell of an enzyme, RNase III, which cleaves the large precursor RNA (that is the product *in vitro*) into mature messengers. Hercules *et al.* (*ibid.*, 840) now report that this cleavage represents a step essential for phage gene expression, since only the mature mRNAs and not their large precursor, are active as templates for translation *in vitro*. This concept is supported by the observation that the early phage genes are only poorly translated in RNase III⁻ mutants of *E. coli*. Why the precursor RNA cannot be translated is obscure; but this system may well prove interesting from the viewpoint of translational control as well as for its processing mechanisms. And although no bacterial systems have yet been shown to suffer precursor maturation as a prerequisite for translation, it is an interesting possibility that RNase III may play this part in uninfected bacteria.

Turning to the transcription of T3 and T7 DNAs which takes place by the phage specified polymerase later during infection, Golomb and Chamberlin (*ibid.*, 769) have mapped the principal units of gene expression. With a template of T7 DNA, the T7 RNA polymerase forms six size classes of mRNA (molecular weights 5.5, 4.5, 2.0, 0.8, 0.4 and 0.2×10^6) equimolar in proportion except for the 2×10^6 species which seems to comprise two classes of mRNA. When the T7 DNA template is shortened by the removal of regions from its right end with nucleases, the smallest mRNA is no longer produced, and the mRNAs of 5.5, 4.5 and (one of) 2.0×10^6 daltons are shortened. Since all these messengers are complementary only to the r-strand of T7 DNA and can be synthesised in spite of deletions at the left end, it seems likely that they are derived from overlapping units of transcription; transcription may start at promotor sites located at 56, 64 and

83% along the phage DNA from its left end, and may end at a common termination site near the right end of the molecule.

When T3 DNA is used as a template for the T7 RNA polymerase, a single major RNA species is formed of 2.0×10^6 daltons. Hybrids between T3 and T7 DNA show various patterns of transcription, consistent with an overlapping arrangement of transcription units. Since hybrid templates can be examined by heteroduplex mapping to define precisely which regions are derived from T3 and which from T7, future use of this technique may allow the start and stop points of the polymerase to be mapped more precisely. There are two classes of late gene in T7, and so it is tempting to speculate that they may be represented by the two classes of mRNA, one comprising the overlapping transcription units and the other corresponding to the remaining messengers. Although previous suggestions have quite often been made that transcription might start at one point and end at any one of several sites to allow coordinate or noncoordinate expression of adjacent genes, the late phage overlapping messengers seem to achieve the same end by reversing the roles of the start and stop points. It remains to be seen whether this has wider significance for the uninfected bacterium.

How algae survive desiccation

from Peter D. Moore
Plant Ecology Correspondent

Few habitats are subjected to the extremes of environmental conditions experienced by the intertidal zones of coasts. Plants and animals living in these regions face wide fluctuation in temperature, insolation, salinity and desiccation with each ebb and flow of the tide. Most of these organisms are essentially marine and therefore it is the period of emersion which represents the stress phase which must be tolerated to ensure survival. The effect of this stress is not laid evenly over the eulittoral zone, but is most acute at its higher levels, where the emersion period is most protracted. It is this variation of stress intensity with vertical height which, in conjunction with the factor of interspecific competition, accounts for the well known intertidal zonation of benthic marine algae (see Zaneveld, *Am. Zool.*, 9, 367; 1969).

Many of the algae of the upper eulittoral suffer emersion periods of several days, sometimes even weeks, during which they become thoroughly desiccated. The physiological mechanisms which enable these morphologically simple plants to survive such

stresses have been a source of interest to botanists for some while.

One aspect of algal physiology which has received attention is that of carbon balance, especially during emersion periods. Chapman (*Proc. 5th Int. Seaweed Symp. Halifax N.S.*, 217; 1966) found that the respiration rate of *Carpophyllum* decreases as desiccation proceeds, falling to 20% of the hydrated rate at 80% desiccation. Bidwell and Craigie (*Can. J. Bot.*, 41, 179; 1963) also demonstrated that carbon fixation is reduced during emersion in *Fucus vesiculosus*. Ogata (*J. Shimonosaki Univ. Fish.*, 16, 89; 1968) has since reported that the depression of respiration rate during desiccation is found in many algae, but that rehydration results in an almost instantaneous resumption of normal respiration.

The current *Carnegie Institution of Washington Year Book*, which covers the years 1972-73, contains information concerning the effect of desiccation on the photochemical reaction of photosynthesis in an intertidal alga. Fork and Hiyama (page 384) have made a study of *Porphyra perforata* and *P. tenera*, species of the upper eulittoral zone. Under normal, hydrated conditions cytochrome *f* is oxidised by light and becomes reduced in darkness. This ceased to happen if the plants had been dried in the sun, which could mean that no electrons were available from the oxidation of water, or possibly that there was a physical uncoupling of cytochrome *f*. Rewetting of the alga immediately resulted in light induced oxidation of cytochrome *f*, indicating that the primary photochemical apparatus had survived the drying process.

The stability of the photosynthetic equipment of *Porphyra* comes as no surprise to botanists who are familiar with this upper shore alga which daily suffers desiccation yet survives. This work does, however, demonstrate most precisely the nature of this stability. This plant's resilience is even more remarkable when one considers its apparently delicate structure; unlike the robust fucoids, *Porphyra* forms sheets of tissue just one cell in thickness. For this very reason *Porphyra* may prove to be an eminently suitable subject for future studies on subcellular responses to extreme environmental conditions.

Correction

IN the News and Views article "Palindromes in eukaryotic DNA" (*Nature*, 248, 733; 1974), the sequence in the third paragraph should read:

5' A B C t C' B' A' 3'
3' A' B' C' t C B A 5'

and not as printed.

Two plates in Africa during the Cretaceous?

Kevine Burk & John F. Dewey

Department of Geological Sciences, State University of New York at Albany, Albany, New York 12222

Plate tectonic theory is based on the idea of the conservation of displacement at the ends of major faults¹: ridges, trenches and triple junctions are linked by transform faults so that a plate boundary may not end except by joining two others at a triple junction. This is a consequence of the motion of torsionally rigid plates in which large permanent strains are restricted to narrow boundary zones. Although brittle intraplate deformation occurs², excellent fits between rifted continental margins³ and between pairs of magnetic anomaly isochrons⁴ show that plates do not generally suffer significant torsional strains.

Provided that the zones in which large, permanent deformations occur are narrow relative to the plates they surround, plate tectonics is a useful theory. The behaviour of wide zones of interplate deformation may be approximated either by 'soft' regions such as the Himalayas and Tibet⁵ or by complex microplate systems such as the present Mediterranean⁶.

It is important to understand to what extent relative plate displacements can be registered as strain in a zone too wide and diffuse to be realistically termed a plate boundary. We describe here a wide zone of strain that developed in Africa during the Cretaceous, recognition of which leads us to the view that Africa, from about 125 Myr ago to about 80 Myr ago, was not behaving as a single rigid plate.

Evolution of the Benue Trough

PLATE accretion began in the South Atlantic about 125 Myr ago, during the late Valanginian–early Hauterivian⁷. Aptian rock salt and carnallite in the Gabon Basin overlie a mainly non-marine sequence and are themselves overlain by marine shales and carbonates (Fig. 1). This provides evidence of a narrow proto–South Atlantic with restricted circulation during late Aptian times⁸. Plate accretion and separation of South America from Africa followed an early Cretaceous phase of intracontinental rifting and magmatism. During this phase rifts were formed, along which plate accretion subsequently occurred. Rifts, some of which contain up to 9 km of sediment, were generated striking at high angles to the continental margin⁹. In Africa and South America, nodal alkaline and peralkaline magmatism and widespread basaltic and rhyolitic volcanism occurred during this rifting phase. The rifting and magmatic activity have been related to the development of domes, crested by rift systems with three branches, which occur at hot spots¹⁰. Plate accretion began on two rift arms, leaving the third as a failed arm or aulacogen¹¹.

The Benue Trough (Fig. 2) is one such rift but it differs from others in that plate accretion occurred along it during early Cretaceous times. Three pairs of magnetic anomalies strike north-eastward beneath the Niger Delta^{12,13}; we suggest that they are late Keathley sequence anomalies developed in Hauterivian–Barremian time during the early opening of the Benue Trough, when a ridge–ridge–ridge triple junction existed in the proto–Gulf of Guinea. The oldest sediments exposed in the Benue Trough are Albian shales (Fig. 1) which pass up

into a sequence of Albian to early Santonian shales with minor Turonian alkali basalts and thick Cenomanian deltaic sequences (Fig. 1). We suggest that this mainly sedimentary sequence was deposited in an already open, or slowly opening, narrow ocean. Near the top of the sequence, late Coniacian andesites are intercalated in shales. The whole sequence was folded during the Santonian (Fig. 1) about axes which strike north-east¹⁴. We interpret the late Coniacian andesites as evidence of subduction of part of the oceanic floor of the Benue Trough, and the Santonian folds as indicating closure of the trough. We are unsure of relative motion, if any, across the Benue Trough during Albian to Turonian times, though the Turonian alkali basalts may indicate very slow extension¹⁵. Twice, therefore, during the Cretaceous evolution of Africa, a plate margin passed into the continent from a triple junction in the Gulf of Guinea, so that there must have been two African plates at these times, one south-east and one north-east of the Benue Trough. To where did these plate margins continue in Africa, and how and over what area was relative displacement between the two African plates expressed as strain?

Cretaceous Africa north-east of Benue Trough

There is no evidence of a discrete well-defined plate margin north-east of the Benue Trough during either the Hauterivian–Barremian extensional phase or the Coniacian–Santonian convergent phase. We will discuss the extensional phase first and then the convergent phase.

Extensional phase. The Benue Trough divides at the Chum and Poli triple junctions to form three rifts¹⁶, one of which, the Poli, continues as a subsurface feature marked by a positive Bouguer anomaly (Fig. 2). Louis¹⁷ modelled this gravity

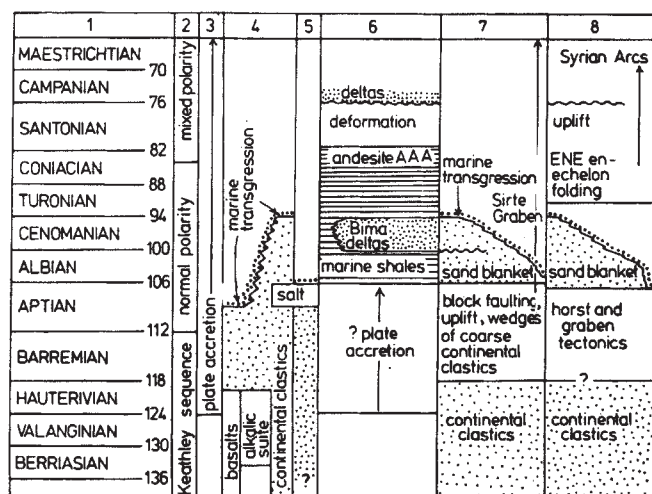


Fig. 1 Correlation chart showing relationships between magmatism, tectonics and sedimentation during the Cretaceous in northern and western Africa and eastern South America. 1, Cretaceous stages; 2, geomagnetic behaviour; 3, plate accretion in South Atlantic (ref. 7); 4, development of eastern continental margin of South America (based mainly on refs 37–39); 5, Gabon Basin (ref. 8); 6, Benue trough (refs 12–15); 7, Algerian and Libyan Sahara (refs 22–26); 8, Egypt and Levant (refs 24, 27). Note that Early Cretaceous magmatism largely predates the development of accreting plate margins and the associated distension of northern Africa.

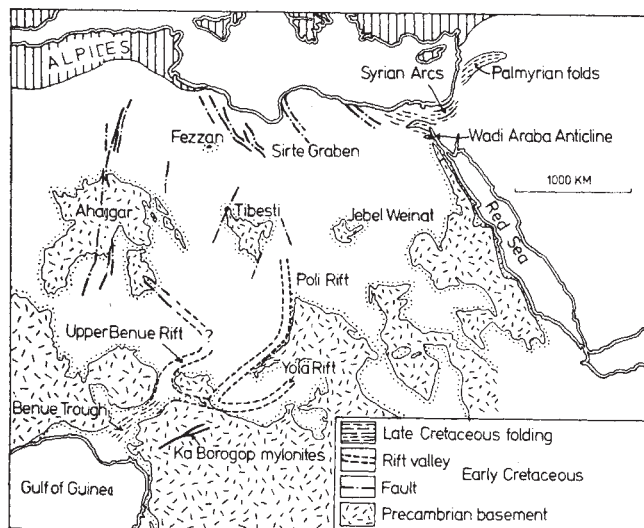


Fig. 2 Northern part of the African continent showing Early Cretaceous rifting and distensional structures and Late Cretaceous folding and compressional structures (based mainly on refs 36, 28 and 17).

anomaly as a basaltic dyke up to 30 km wide. Burke and Whiteman¹⁸ interpreted it as one of the axial dykes related to extension, which are becoming widely known in rift valleys¹⁸⁻²¹. The Upper Benue and Yola intracontinental rifts contain Cenomanian continental sandstones equivalent to the Bima deltas of the Benue Trough that prograded south-westwards along the narrow Benue Ocean. From the Berriasian until the Hauterivian, most of North Africa was covered by a thin veneer of continental deposits. During the Barremian–Aptian, block faulting and differential regional uplift affected a wide region of North Africa from the Algerian Sahara to Egypt²² (Figs 1, 2 and 3B). Most of the block faulting took place on reactivated basement faults trending roughly north-south. Tibesti was uplifted and shed sand wedges²³, and north-south block faulting in the Ahaggar was accompanied by local spreads of coarse clastics in graben, and erosion or nondeposition over horsts²⁴. The Jebel Weinat Massif was also rising at this time²⁵. In north-west Libya, gentle folding of early Cretaceous rocks occurred before the deposition of late Aptian sediments²⁶ and, in the Fezzan, Cenomanian sediments rest unconformably on older Cretaceous rocks. These strong differential movements over the Algerian Sahara and Libya, ceased by Albian times. An Albian sandstone sheet of fairly uniform thickness completely covers the Barremian–Aptian structures²⁴. The Sirte graben complex of northern Libya (Fig. 2) controlled sedimentation during the middle and late Cretaceous. In Egypt, faults trending north-west were active during Barremian–Aptian times, producing a series of graben and horsts with up to 2 km of shale. The Barremian–Aptian activity suggests that a large region of northern Africa (Fig. 2) was slowly distending, between two African plates, in response to the same displacement that was being accommodated by plate accretion in the Benue Trough.

Compressional phase. The Cretaceous deformation of the Benue Valley is dated as Santonian (Fig. 1). Deformation was also occurring elsewhere in northern Africa at this time²⁴. The Syrian Arcs, the Wadi Araba anticline and related structures (Fig. 2) began to form late in the Turonian and grew during the Coniacian and Santonian (Fig. 1). An important sub-Campanian unconformity records this deformation phase in Egypt (Fig. 1). In the Levant, the Santonian rests directly on gently folded Turonian and older rocks²⁷. The Ka Borogop mylonite belt (Fig. 2) is not Precambrian, as was previously thought, but affects Cretaceous conglomerates²⁸. Its upper age limit is unknown but we here suggest that it was developed, during the Coniacian–Santonian closing of the Benue Trough, perhaps

with a significant strike-slip component (compare with ref. 29). Therefore, although convergence of the two African plates was accommodated by subduction in the Benue Trough it was apparently taken up by intracontinental compression over at least part of the region of northern Africa affected by Barremian–Aptian distension. Although the relative displacement of the two plates may not have been large we are unable to determine its magnitude or precise direction. The Benue Trough was a structure which narrowed towards the continent during the early Cretaceous and all early Cretaceous strain between the Benue Trough and Egypt seems to have been extensional. This suggests that the pole of relative motion between the two African plates at this time lay north-east of Egypt. In plate tectonic terms, the broad zone of extension may be regarded as either a plate boundary up to 2,000 km wide or as a series of smaller intracontinental plates. If nothing were known of the history of the Benue Trough this extensional zone might be regarded as an enigmatic zone of intraplate, intracontinental rifting.

Stresses unrelated to latitudinal motion

Intraplate earthquakes, well away from possible plate boundary effects, yield dominantly thrust fault and strike-slip first motion solutions on all present major plates, indicating that plates are undergoing local, brittle deformation in response to compressive stresses³. There is no clear pattern of compression directions, but compression has been deduced at localities from latitudes of about 60°N to 40°S. Intraplate extension has been deduced only for eastern Baffin Island and southern Africa.

We have argued¹⁰ that intraplate extension which yields intracontinental rift-valley systems is related to the growth of hot spot domes and periclines. As the domes grow, they are characterised by alkaline and peralkaline magmatism and eventually by crestral rifting, commonly in three-armed systems. This hot spot phase of intracontinental rifting and magmatism may be protracted and abortive but it may lead to a phase in which rift valleys propagate and link up, followed by continental fragmentation and the opening of oceans by plate accretion³⁰. Burke and Wilson³¹ have argued that extensive hot spot activity before continental separation may indicate

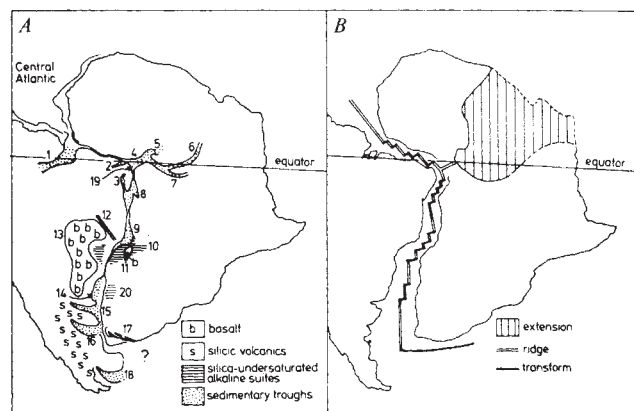


Fig. 3 A: Berriasian–Valanginian magmatism, tectonics and sedimentation on the site of the future South Atlantic. 1, Marajo basin (ref. 37); 2, Potiguar basin (ref. 37); 3, Jucano basin (ref. 37); 4, Benue trough (ref. 12); 5, Upper Benue rift (ref. 16); 6, Poli rift (ref. 17); 7, Yola rift (ref. 16); 8, Sergipe-Gabon basin (ref. 39); 9, Cuanza basin (ref. 40); 10, Angola alkaline province (ref. 41); 11, Kaokoveld basalts (ref. 42); 12, Goiana arch (refs 37 and 38); 13, Parana basalts (ref. 37); 14, Pelotas basin (ref. 37); 15, Rio Salado basin (ref. 9); 16, Bahia Blanca basin (ref. 9); 17, Algoa and associated Cape Province basins (ref. 43); 18, Malvinas basin (ref. 9); 19, Cabo granite and associated silicic alkaline rocks (ref. 37); 20, Messum alkaline province (ref. 44). B: Apatian plate boundaries in the South Atlantic and Africa. The circle with a dot lies in the general area of a pole describing the Apatian motion of north-western Africa with respect to south-eastern Africa.

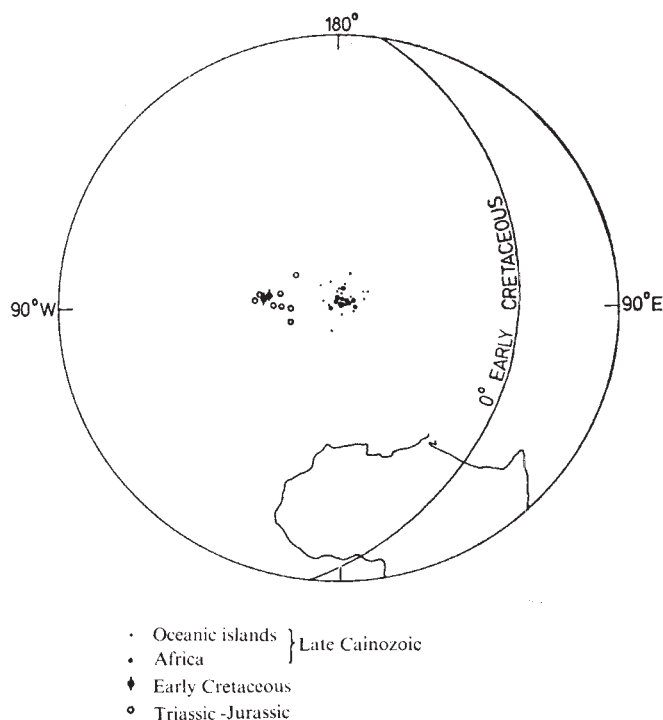


Fig. 4 Palaeomagnetic poles for the African plate (refs 33 and 34) form two groups marking times in the Mesozoic and Tertiary when the plate was at rest with respect to the spin axis. Between about 100 and 25 Myr ago African plate motion with respect to the main dipole field was approximately a 30° rotation about a pole in the Gulf of Guinea. Rifting and igneous activity dominated the continent while it was at rest and were minimal while it was moving.

that the continent, or plate, in question was stationary or moving very slowly with respect to underlying mantle plumes. Once plate accretion between two continental fragments begins, hot spot activity may diminish or cease on one or both of the fragments; if motion relative to the plume population is fast. Our model of rift valley generation involves a hot spot phase with dominantly vertical movements and only mild extension, followed by a phase of extension leading to continental break up.

Turcotte and Oxburgh³², using membrane stress theory, have adopted a different view of intraplate deformation. They argue that as plates, considered as thin elastic membranes, change their radius of curvature as they change latitude, stresses build up. If, for instance, a portion of a plate were moving to a lower latitude at a rate of 1 cm yr^{-1} , then membrane stresses would equal the tensile strength of the lithosphere after 30 Myr, provided that the stresses were not dissipated. An increase in latitude of 90° would, however, be required before membrane stresses were about equal to the compressive strength of the lithosphere. Membrane stresses thus cannot account for the widespread intraplate compressive failure of the lithosphere² although they could, theoretically, generate tensional failure to form rift valleys. If intracontinental extension and rifting results from the propagation of cracks developed in response to membrane stresses, extension should predate magmatism. In rift valleys generated by hot spots, magmatism should predate significant extension. We here show that the early Cretaceous tectonic-magmatic history leading to the separation of Africa and South America and the opening of the Benue Trough favours the hot spot interpretation.

From about 160 to 120 Myr ago, magmatism was widespread in the general region along which Africa separated from South America (Fig. 3A). Alkaline and peralkaline igneous rocks were intruded and extruded in distinct nodes (Cabo Granite, Rio de Janeiro, Messum); basalts (Parana, Kaokoveld) and rhyolites (southern Argentina) formed extensive volcanic

provinces. During and after this magmatic phase, great thicknesses of non-marine Valanginian to Barremian sediments accumulated in troughs with faulted boundaries (rifts and aulacogens) parallel and at high angles to the future continental margins of South America and Africa. The beginning of plate accretion in the South Atlantic overlapped the waning phases of magmatism and aulacogen formation (Fig. 1) yet nonmarine sedimentation persisted until middle to late Aptian times. The sea had invaded the Benue Trough by earliest Albian times and it is likely that the Benue Trough opened during the extension of North Africa (Figs. 1 and 3B). Significant extension leading to the separation of South America and Africa, the opening of the Benue Trough and the distension of North Africa, postdated most of the early Cretaceous magmatic-aulacogen phase.

Late Triassic to early Cretaceous palaeomagnetic poles for Africa³³ cluster closely around a mean at 265°E , 65°N (Fig. 4). While Gondwana was breaking up there were no systematic changes to lower latitudes of the kind needed for an explanation in terms of membrane stress of the associated early Mesozoic rift systems. Africa, during approximately the last 25 Myr of its history, has been characterised by a similar episode of hot spot uplift, volcanism and rifting. African palaeomagnetic poles for this time interval^{33,34} similarly cluster around a mean near the present pole (Fig. 4). Rifting and magmatism in Africa have developed at times when Africa did not change its latitude. Between 110 and 25 Myr ago Africa changed its position, relative to the spin axis, in a way that may be approximated by an anticlockwise finite difference rotation of 30° around a pole at 0°N , 10°W (Fig. 4). During this time there was little or no rifting or magmatism on the African continent.

The closing of the Benue Trough and the compression of North Africa occurred at a time of major changes in relative plate motion. As a result of the opening of the Labrador Sea, the relative motion of Africa with respect to Europe changed to an anticlockwise rotation about a pole at 25.4°N , 163.8°E (ref. 35). Plates moving with respect to underlying plumes may generally be in compression whereas those that are at rest with respect to underlying plumes develop tensional features related to hot spot activity.

Received December 13, 1973; revised February 19, 1974.

- ¹ Wilson, J. T., *Nature*, **207**, 343 (1965).
- ² Sykes, L., and Sbar, M., *Nature*, **245**, 298 (1973).
- ³ Bullard, E., Everett, J. E., and Smith, A. G., *Phil. Trans. R. Soc.*, **258**, 41 (1965).
- ⁴ Pitman, W. C. III, and Talwani, M., *Bull. geol. Soc. Am.*, **83**, 619 (1972).
- ⁵ Dewey, J. F., and Burke, K., *J. Geol.*, **81**, 683 (1974).
- ⁶ McKenzie, D., *Nature*, **226**, 239 (1970).
- ⁷ Larson, R. L., and Ladd, J. W., *Nature phys. Sci.*, **246**, 209 (1973).
- ⁸ Belmonte, Y., Hirtz, P., and Wenger, R., in *Salt Basins around Africa*, (edit. by Ion, D. C.), 55 (Institute of Petroleum, London, 1965).
- ⁹ Ludwig, W. J., Ewing, J. I., and Ewing, M., *Bull. Am. Ass. Petrol. Geol.*, **52**, 2337 (1968).
- ¹⁰ Burke, K., and Dewey, J. F., *J. Geol.*, **81**, 406 (1973).
- ¹¹ Hoffman, P., Dewey, J. F., and Burke, K., in *Modern and Ancient Geosynclinal Sedimentation*, (edit. by Dott, R. H., and Shaver, R. H.), (Society of Economic Palaeontologists and Mineralogists, Tulsa, in the press).
- ¹² Burke, K., Dessauvage, T. F. W., and Whiteman, A. J., *Nature phys. Sci.*, **233**, 51 (1971).
- ¹³ Burke, K., Dessauvage, T. F. W., and Whiteman, A. J., in *African Geology, Ibadan, 1970* (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 187 (University of Ibadan, Ibadan, 1972).
- ¹⁴ Murat, R. C., in *African Geology, Ibadan, 1970* (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 251 (University of Ibadan, Ibadan, 1972).
- ¹⁵ Carter, J. D., Barber, W., Tait, E. A., and Jones, G. P., *Bull. geol. Surv. Nigeria*, **30**, (1963).
- ¹⁶ Burke, K., and Whiteman, A. J., in *Implications of Continental Drift to the Earth Sciences*, 2 (edit. by Tarling, D. H., and Run-corn, S. K.), 727 (Academic Press, London, 1973).
- ¹⁷ Louis, P., *Contribution géophysique à la connaissance géologique du bassin du lac Tchad* (Mémor de la Organisation de la Recherche Scientifique dans les Territoires d'outre Mer, n° 42, Paris, 1970).

- ¹⁸ Griffiths, D. H., King, R. F., Khan, M. A., and Blundell, D. J., *Nature phys. Sci.*, **229**, 69 (1971).
- ¹⁹ Searle, R., and Gouin, P., *Tectonophysics*, **15**, 279 (1972).
- ²⁰ Ocola, L., and Meyer, R. P., *J. geophys. Res.*, **78**, 5173 (1973).
- ²¹ Ramberg, I., *Nature phys. Sci.*, **240**, 149 (1972).
- ²² Karpoff, R., *Bull. Soc. geol. Fr.*, Ser. 7, **7**, 469 (1965).
- ²³ Lelubre, M., *Bull. Soc. geol. Fr.*, Ser. 5, **19**, 251 (1949).
- ²⁴ Sander, N. J., in *Geology and History of Sicily*, (edit. by Alvarez, W., and Gohrbandt, K. H. A.), 43 (Petroleum Exploration Society of Libya, 1970).
- ²⁵ Kiltzsch, E., in *African Geology, Ibadan, 1970* (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 423 (University of Ibadan, Ibadan, 1972).
- ²⁶ Busson, G., in *Guidebook to the Geology and History of Tunisia*, 131 (Petroleum Exploration Society of Libya, 1967).
- ²⁷ Bendor, Y. K., Grader, P., Parnes, A., Reiss, Z., Shiftan, Z., and Vroman, A., (*Lexique Stratigraphique International*, **3**, 8 (1960)).
- ²⁸ Le Marechal, A., and Vincent, P. M., in *African Geology, Ibadan, 1970*, (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 229 (University of Ibadan, Ibadan, 1972).
- ²⁹ Fitch, T. J., *J. geophys. Res.*, **77**, 4432 (1972).
- ³⁰ Dewey, J. F., and Burke, K., *Geology*, **2**, 57 (1974).
- ³¹ Burke, K., and Wilson, J. Tuzo, *Nature*, **239**, 387 (1972).
- ³² Turcotte, D. L., and Oxburgh, E. R., *Nature*, **244**, 337 (1973).
- ³³ McElhinny, M. W., *Palaeomagnetism and Plate Tectonics* (Cambridge University Press, Cambridge, 1973).
- ³⁴ Piper, J. D. A., and Richardson, A., *Geophys. J. R. astr. Soc.*, **29**, 147 (1972).
- ³⁵ Dewey, J. F., Pitman, W. C. III, Ryan, W. B. F., and Bonnin, J., *Bull. geol. Soc. Am.*, **84**, 3137 (1973).
- ³⁶ *Tectonic Map of Africa, 1:5M* (Association des Services Geologiques Africains—UNESCO, Paris, 1968).
- ³⁷ Almeida, F. F. M., *Twenty-fourth International Geological Congress, Montreal*, **3**, 339 (1972).
- ³⁸ Neill, W. M., *Nature phys. Sci.*, **245**, 104 (1973).
- ³⁹ Wardlaw, N. C., and Nicholls, G. D., *Twenty-fourth International Geological Congress, Montreal*, **6**, 43 (1972).
- ⁴⁰ Brognon, G. P., and Verrier, G. R., *Bull. Am. Ass. Petrol. Geol.*, **50**, 108 (1966).
- ⁴¹ Rodrigues, B., in *African Geology, Ibadan, 1970*, (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 149 (University of Ibadan, Ibadan, 1972).
- ⁴² Siedner, G., and Miller, G. A., *Earth planet. Sci. Lett.*, **4**, 451 (1968).
- ⁴³ Rigassi, D. A., and Dixon, G. E., in *African Geology, Ibadan, 1970*, (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 513 (University of Ibadan, Ibadan, 1972).
- ⁴⁴ Marsh, J. S., *Earth planet. Sci. Lett.*, **18**, 317 (1973).

Repeating sequences in aminoacyl-tRNA synthetases

G.L.E. Koch, Y. Boulanger* & B.S. Hartley

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

Some aminoacyl-tRNA synthetases have elongated by fusing two very similar sequences.

AMINOACYL-tRNA synthetases are responsible for the specificity of translation of the genetic code. They each catalyse a similar reaction in which a particular amino acid reacts with ATP probably forming a 5'-aminoacyl adenylate, which then transfers to the 3'-terminal adenosine of a particular tRNA, eliminating AMP. Their specificities are also similar in that different tRNAs have similar structures¹ and that each enzyme requires an unsubstituted α -amino acid and ATP for the complete reaction.

To avoid errors in protein synthesis, however, they must be completely specific for a particular amino acid and a particular tRNA. Since the genetic code seems to be universal, this specificity has been conserved over a very long period. We might therefore expect these enzymes to be a family with homologous primary and tertiary structures that are under enormous selective pressure to conserve their essential features.

The accumulation of data on the protomeric units of several synthetases has revealed an objection to this view, however. Broadly, four classes of synthetases can be defined on the basis of protomer size and binding capacity. (1) Those containing a single polypeptide chain with a molecular weight of about 120,000 and containing one binding site for amino acid and tRNA, for example, isoleucyl-tRNA synthetase from *Escherichia coli*^{2,3}. (2) Those containing two identical protomers each with a molecular weight of about 90,000 and possessing one binding site for amino acid and tRNA per protomer, for example methionyl-tRNA synthe-

tase from *E. coli* (ref. 4, and G.K., unpublished). (3) Those containing two identical protomers each with a molecular weight of about 40,000, for example, tryptophanyl-tRNA synthetase from *E. coli*⁵. (4) Those containing dissimilar protomers, for example phenylalanyl-tRNA from yeast⁶, glycyl-tRNA synthetase from *E. coli*⁷. This wide diversity in the sizes and organisation of the protomers does not seem consistent with the concept of evolution from a common precursor since the known classes of homologous protein generally contain protomers of very similar size. These apparent inconsistencies have led us to isolate several aminoacyl-tRNA synthetases from a single source, the thermophile *Bacillus stearothermophilus*, and to search for sequence homologies between them.

Enzymes from *B. stearothermophilus*

Aminoacyl-tRNA synthetases are present in low amounts in most cells and many of them are denatured during the lengthy purification that is necessary. The thermophilic organism, *B. stearothermophilus* was chosen as a source because proteins from this species exhibit remarkable stability. A purification procedure that yields about 100 mg of pure leucyl-, methionyl-, tryptophanyl-, tyrosyl- and valyl-tRNA synthetases from 5 kg wet weight of these cells has been developed in collaboration with the Microbiological Research Establishment, Porton Down, Wilts. All enzyme samples used in these studies were more than 95% pure.

Before determining any of the chemical parameters of these enzymes their subunit structures had to be established. The molecular weights of the native proteins determined by gel filtration⁸ were usually sufficiently accurate (Table 1). On the other hand, it was essential to estimate the sizes of the protomers on proteins which had been completely de-

*Present address: Universite Louis Pasteur, Laboratoire de Biochimie, Rue R. Descartes, 67 Strasbourg, France

Table 1 Subunit structures of *B. stearotherophilus* synthetases

Aminoacyl-tRNA synthetase	Molecular weight (native)	Molecular weight (subunit)	Subunit structure
Tryptophanyl-	70,000 (E, F)	35,000 (S, G)	2 × 35,000
Tyrosyl-	95,000 (F)	44,000 (S)	2 × 44,000
Methionyl-	135,000 (E, F)	66,000 (S, G)	2 × 66,000
Valyl-	100,000 (F)	110,000 (S, A)	1 × 110,000
Leucyl-	100,000 (E)	110,000 (S, A)	1 × 110,000

E, Equilibrium sedimentation; F, Sephadex gel filtration; G, equilibrium sedimentation in 6 M Guanidine hydrochloride; S, SDS gel electrophoresis; A, agarose gel filtration in 0.1 % SDS.

natured and dissociated. For this purpose the proteins were either boiled in 1 % SDS or preoxidised with performic acid and boiled in 1 % SDS. The molecular weights of the protomers were determined by electrophoresis in SDS-polyacrylamide gels⁹ or by gel filtration on columns of SDS-agarose (Table 1). Investigations of the tryptophanyl-enzyme were hampered by autolysis in the stored preparations. The size of the protomers in methionyl-tRNA synthetase was confirmed by equilibrium ultracentrifugation in the presence of 6 M guanidine hydrochloride¹⁰. The results show that valyl- and leucyl-tRNA synthetase are monomeric proteins

Table 2 Amino acid compositions of *B. stearotherophilus* synthetases

Amino acid	Aminoacyl-tRNA synthetase			
	Tyrosyl-	Methionyl-	Valyl-	Leucyl-
Cyst†	2	5	8	6
Asp	29	56	91	93
Thr	23	27	52	50
Ser	18	24	48	33
Glu	44	69	117	120
Pro	6	17	26	30
Gly	33	50	76	72
Ala	33	44	91	67
Val	23	41	80	64
Met	11	18	32	33
Ile	26	39	66	51
Leu	41	54	97	77
Tyr	17	29	30	29
Phe	18	22	38	34
Trp‡	11	19	19	24
His	9	12	31	26
Lys	27	45	68	65
Arg	28	34	69	47
Molecular weight	45,000	66,000	110,000	110,000

* Calculated on the basis of the molecular weights of the protomers.

† From analyses of the oxidised protein, using oxidised chymotrypsinogen as standard. This procedure can give over estimates of up to 15 % for cysteic acid.

‡ Estimated by the method of Edelhoch¹⁷.

with molecular weights of about 110,000 whilst tyrosyl- and methionyl-tRNA synthetase are dimers whose protomeric units have molecular weights of 45,000 and 66,000 respectively.

To facilitate the discussion the amino acid compositions of the proteins shown in Table 2 have been calculated on the basis of the molecular weight of the protomers present in each protein. Since the cysteine (cysteic acid) contents of the enzymes proved small, special precautions were taken to ensure the accuracy of its analyses. Hydrolysates of chymotrypsinogen and *B. amyloiquefaciens* RNase¹¹ (which contains no cysteine) were used for calibration and the values shown are the averages of several analyses.

Repeating sequences

The first indication that some of the protomers might contain repeating sequences was found in maps of the tryptic peptides but the most convincing evidence for their existence came from estimates of the molar yields of several tryptic peptides from each of the proteins. It was assumed that yields greater than 1 mol of peptide per mol protomer would prove the existence of at least two copies of the

corresponding sequence in the intact protomer. Since the approach relies on the isolation of pure peptides in high yield, certain precautions had to be taken. Preliminary experiments had shown that trypsin digestion was more complete when the proteins were denatured by oxidation rather than carboxymethylation. The trypsin was purified on trypsin inhibitor-Sepharose columns in order to minimise nontryptic cleavages in the digest, and wherever possible only those peptides which were pure after a single purification step were considered since we could not correct for losses during purification.

The tryptic digest of oxidised methionyl-tRNA synthetase was fractionated by ion-exchange chromatography on Locarte L1/5 resin using a gradient of pyridinium-acetate for elution¹². This procedure (Fig. 1) resolved several peptides which proved pure by paper electrophoresis, paper chromatography and amino acid analysis. The amino acid analyses also gave their compositions and yields. The molar yield of each peptide calculated from them was then used to determine the mol peptide per mol of protomer (Table 3). A similar experiment was performed with tryptic digests of oxidised leucyl-tRNA synthetase using a combination of SP-Sephadex chromatography and Sephadex G-50 gel filtration to purify the peptides. This procedure was preferred to the one used with methionyl-tRNA synthetase because it selectively purified the larger acidic peptides in high yield (Table 3).

The yields of tryptic peptides from valyl-tRNA synthetase were estimated by a different procedure. The protein was carboxymethylated with ¹⁴C-iodoacetate¹³, digested with trypsin and the radioactive peptides purified by paper electrophoresis and chromatography. By using radioactive peptides losses during purification could be corrected. Note that the yields (Table 3) have been calculated from the amino acid analyses of the purified peptides and not from the yield of radioactivity which is an unreliable criterion. Nevertheless, the radioactive label was useful in enabling corrections for losses to be made at each step in purification.

The results show that the protomers of methionyl-, valyl- and leucyl-tRNA synthetase gave several peptides with yields between one and two per protomer (Table 3).

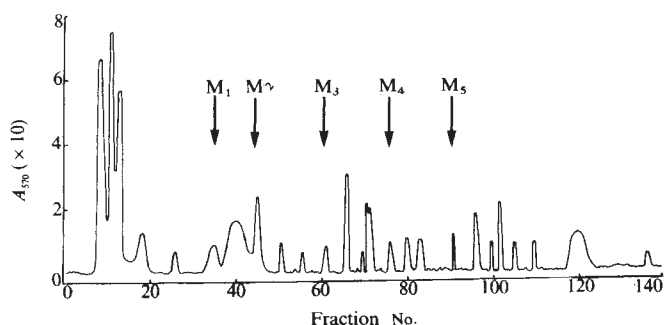


Fig. 1 Separation of the tryptic peptides from methionyl-tRNA synthetase. Enzyme (26 mg) was oxidised with performic acid, digested with trypsin (1:100, w/w) for 4 h at 37° C in 0.5 % NH₄HCO₃ and lyophilised. The peptides were dissolved in 0.2 M pyridine-acetate pH 3.1 and applied to a column (1 cm × 60 cm) of Locarte L1/5 resin equilibrated with the same buffer. The peptides were eluted with a gradient of pyridine-acetate

175 ml 0.2 M pyridine-acetate pH 3.1

350 ml 2 M pyridine-acetate pH 5.0

One-sixth of the effluent was monitored automatically for peptides by the ninhydrin reaction after alkaline hydrolysis, and the rest collected in fractions which were pooled on the basis of the elution profile. Each subfraction was tested for homogeneity by paper electrophoresis at pH 6.5 and 2.1. The material from each of the arrowed peaks contained only one peptide.

The relatively low yields for the peptides from methionyl-tRNA synthetase are probably a result of losses during purification. All the same, the yields provide strong evidence for more than one copy of each of those peptides in the protomer.

Distribution of repeated sequences

The distribution of the repeated sequences might indicate how the proteins have evolved, but would seem to need knowledge of the entire primary structures. We have attempted a short cut which relies on splitting each protomer into a few large fragments and then surveying the fragments for certain peptides. If a peptide is contained within more than one large fragment then the repeating units which make up the protomer are large. If it is contained within one fragment, then the repeated regions must be relatively short and close to one another.

This approach has proved to be useful with valyl-tRNA synthetase. The native enzyme consists of a single polypeptide chain of about 1,000 residues and is extremely sensitive to trypsin which splits the chain into two fragments of unequal size (Fig. 2). The electrophoretic behaviour of the split protein is identical to that of the native enzyme showing that degradation is not extensive. The larger fragment,

Table 3 Estimation of the yields of some tryptic peptides from the synthetases

Protein sample	Peptide + composition	Yield (nmol)	Mol peptide per mol protomer
Methionyl-tRNA synthetase (210 nmol protomer)	M ₁ D T S E G	290	1.39
	I F R		
	M ₂ E P G L K	260	1.25
	M ₃ D E A K	240	1.14
	M ₄ E A R	240	1.14
	M _f M S K	240	1.14
	L ₁ D ⁵ T ₃ S E I P ₂	140	1.55
	G ₃ A ₂ V ₃ I L ₂		
Leucyl-tRNA synthetase (90 nmol protomer)	K R	160	1.78
	D ₂ K	150	1.67
	L ₃ D E G V K	150	1.67
	L ₄ D ₂ S E ₃ A L		
	K		
	V ₁ C T E ₂ G Y	128	1.91
	R		
Valyl-tRNA synthetase (67 nmol protomer)	V ₂ C E A V K	120	1.79
	V ₃ C D ₂ E P G	120	1.79
	A L		

The peptides from methionyl-tRNA synthetase were purified according to the procedure shown in Fig. 1. The peptides from leucyl-tRNA synthetase were isolated from a tryptic digest of the oxidised enzyme by passage of the digest through columns of SP-Sephadex C25 equilibrated with 0.17 M pyridine-acetate pH 4.7 and Sephadex G50 equilibrated with 1% pyridine. The peptides from valyl-tRNA synthetase were isolated from a tryptic digest of the ¹⁴C-carboxymethylated protein. The digest was subjected to paper electrophoresis at pH 6.5 and pH 2.1 (see Fig. 2), which completely separated the radioactive carboxymethylcysteine peptides. The peptides were eluted with 1 M ammonia and purified to homogeneity by paper chromatography. The pure peptides were then analysed after hydrolysis and the yield of each calculated. The yield was corrected for losses during purification by comparing the amount of radioactivity in each pure peptide with that present in the same peptide in the unfractionated digest. The single letter notation used for amino acids is that proposed in ref. 18.

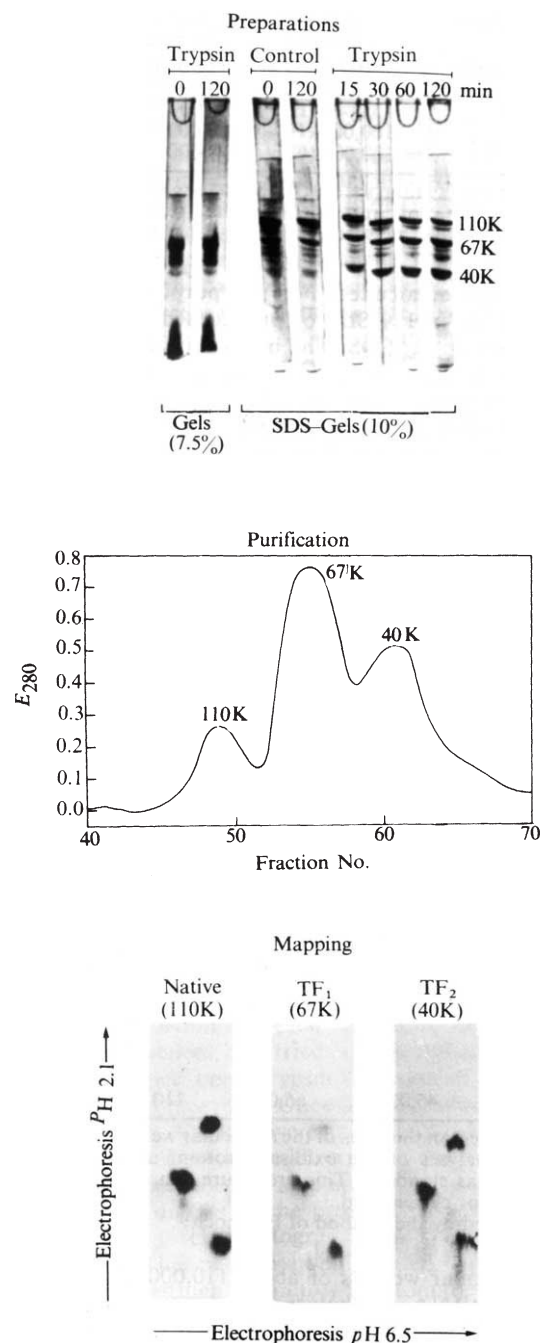


Fig. 2 The distribution of the carboxymethylcysteine sequences between the fragments of valyl-tRNA synthetase. Enzyme (40 mg in 20 ml of 0.1 M Tris-HCl pH 7.5) was incubated with trypsin (1:2500 w/w) at 37° C for 2 h. Aliquots were removed at various intervals and subjected to polyacrylamide gel electrophoresis in the presence and absence of SDS. During incubation the native enzyme was split into two fragments with molecular weights of about 67,000 and 40,000. The digested enzyme was made 2% in SDS and 2% in 2-mercaptoethanol, immersed in boiling water for 5 min and passed through a column of agarose 1.5 M equilibrated with 100 mM Tris-HCl, 0.1% SDS and 10 mM 2-mercaptoethanol. Material from the 67K and 40K peaks was pooled and recycled to yield homogeneous preparations. Samples of the native enzyme, valyl-TF₁ (67K) and valyl-TF₂ (40K) were reduced and carboxymethylated with ¹⁴C-iodoacetate in 6 M guanidine hydrochloride, digested with trypsin (1:100 w/w) in 0.5% NH₄HCO₃ for 4 h at 37° C and the soluble peptides subjected to two-dimensional mapping by paper electrophoresis. The regions of the maps containing the major radioactive peptides from each sample are shown. The apparent inhomogeneity of the sample is due to considerable overloading of the gels.

valyl-TF₁ (molecular weight 67,000), and the smaller, valyl-TF₂ (molecular weight 40,000), were dissociated and separated from one another by gel filtration on columns of agarose 1.5 M equilibrated with 0.1% SDS. The amino acid analyses show eight half cystines in the native protomer, four half cystines in the large fragment and three half cystines in the small fragment¹⁴. The three proteins were reduced, ¹⁴C-carboxymethylated, digested with trypsin and subjected to two dimensional mapping. Autoradiographs of the maps were prepared to identify the carboxymethylcysteine peptides (Fig. 2). The same radioactive peptides stand out in all three maps showing that both fragments contain the same carboxymethylcysteine sequences. This means that the repeated peptides are distributed between widely separated regions of the protomer and that it is made up of long repeating units and not short, clustered repeats.

When digested with low concentrations of subtilisin, methionyl-tRNA synthetase is converted into a fully active fragment which is composed of a single polypeptide chain with a molecular weight of about 55,000. Methionine is the amino terminus of both the native enzyme and the fragment, indicating that the trypsin digestion has removed a segment of about 100 residues from the C-terminus of the native protomer. The differences between the amino acid analyses of the native enzyme and the fragment show that the excised region contains five lysine and 11 arginine residues and should therefore yield 17 tryptic peptides. Except for the relative intensities of some of the spots, however, two-dimensional maps of the tryptic peptides from the two proteins display almost complete homology (Fig. 3). Such a result would be expected if the native protomer were composed of two long homologous sequences fused end to end, digestion of the excised region from the C-terminus having removed one copy of the duplicated peptides and left the qualitative features of the fingerprint unchanged.

Homology of the repeated sequences

To find the degree of homology of the repeating units and the proportion of each protomer made up by the repeating sequences, we tried to determine the number of peptides obtained upon trypsin digestion of each protein by high resolution mapping. Once again oxidised proteins were used since they did not give significant amounts of insoluble material after digestion with trypsin, making nearly all of the protein amenable to mapping.

The tryptic digests of the proteins were first fractionated by ion-exchange chromatography on a column of Locarte L1/5 resin. The peptides were eluted with a linear pH gradient, the effluent monitored automatically by the ninhydrin reaction and fractions collected. The fractions containing the material in each of the ninhydrin-positive peaks were pooled, concentrated and each peak subjected to two-dimensional mapping by paper electrophoresis at pH 6.5

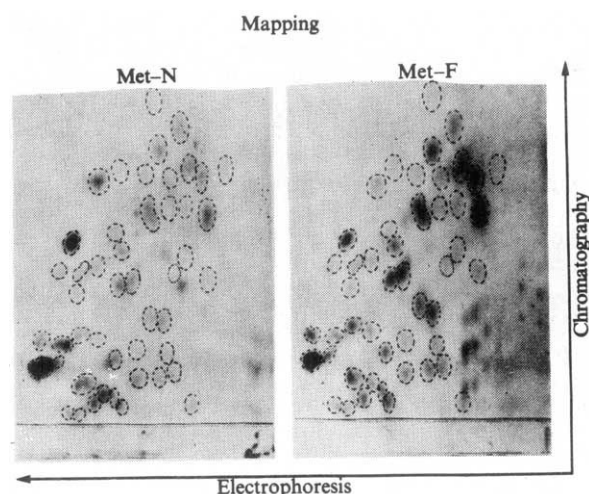


Fig. 3 Two-dimensional maps of the tryptic peptides from methionyl-tRNA synthetase and its subtilisin fragment. The fragment was prepared from the native enzyme by digestion with subtilisin (1:2500 w/w) in 100 mM Tris-HCl pH 7.5, and purified by gel filtration on Sephadex G150. Samples of the homogeneous fragment and the native enzyme were oxidised with performic acid, digested with trypsin (see legend to Fig. 1) and the soluble tryptic peptides (over 95% of the proteins used) subjected to paper electrophoresis (formic acid-acetic acid buffer pH 2) and paper chromatography (butanol-acetic acid-water-pyridine 30:6:24:20). Maps were stained with 1% ninhydrin in acetone.

and 2.0. The results obtained with methionyl-, valyl- and leucyl-tRNA synthetase showed that this procedure fractionates the peptides in the digests very well and that the chance of low estimates of the number of tryptic peptides as a result of coincidence is negligible. Table 4 summarises the estimates of the number of tryptic peptides from each protein. Table 4 also shows estimates of the number of arginine and histidine peptides which were determined from two-dimensional maps of the tryptic peptides using the specific stains for these residues. Estimates of the number of cysteine peptides were obtained from studies on tryptic digests of the ¹⁴C-carboxymethylated proteins. The number of carboxymethylcysteine peptides among the soluble proteins was determined after fractionation by ion-exchange chromatography followed by paper electrophoresis of the radioactive fractions (Fig. 4). Two radioactive peaks were obtained from methionyl- and leucyl-tRNA synthetase and electrophoresis showed that each contained a single radioactive peptide. Paper electrophoresis of the single radioactive peak obtained from valyl-tRNA synthetase showed two radioactive peptides of equal intensity. The recovery of radioactivity after ion-exchange chromatography exceeded 70% of that applied to the column which excludes the possibility of significant losses of radioactive peptides during

Table 4 Estimates of the number of tryptic peptides

Aminoacyl-tRNA synthetase	Total peptides		Arginine peptides		Histidine peptides		Cysteine peptides	
	Expected*	Observed	Expected*	Observed	Expected*	Observed	Expected*	Observed
Tyrosyl-	56	45-50	28	22	9	10	2	2
Methionyl-	80	40-45	34	19	12	6	5	3
Valyl-	138	50-55	69	32	31	12	8	3
Leucyl-	113	40-45	47	24	26	10	6	2-3

* Calculated from the amino acid analyses shown in Table 2.

The total number of tryptic peptides obtained from each protein was determined from digests of the oxidised proteins. The soluble peptides, comprising over 90% of the protein used for each digest, were first fractionated according to the procedure described in Fig. 1. The material in each peak was then subjected to two-dimensional mapping on paper, the peptides stained with ninhydrin and the number obtained from all the maps used to estimate the total number of tryptic peptides present in the digest. The number of arginine and histidine peptides was estimated by staining two dimensional maps on paper with *o*-phenanthroline and diazotised sulphanilic acid respectively. Estimates of the number of cysteine-containing peptides were determined from tryptic digests of the ¹⁴C-carboxymethyl proteins (see Fig. 4).

fractionation. The insoluble peptides from methionyl-, valyl-, and leucyl-tRNA synthetase contained 35%, 20% and 5% of the total radioactivity in the carboxymethylated proteins, respectively. They were solubilised by digestion with thermolysin and subjected to paper electrophoresis. A single radioactive peptide was obtained from each of the insoluble fractions and comparisons with thermolysin digests of the soluble peptides indicated that they were unique. It was concluded that there are no more than three cysteine sequences in each protein of which only two of the three from leucyl-tRNA synthetase can be treated as major peptides. The minor peptide from leucyl-tRNA synthetase is

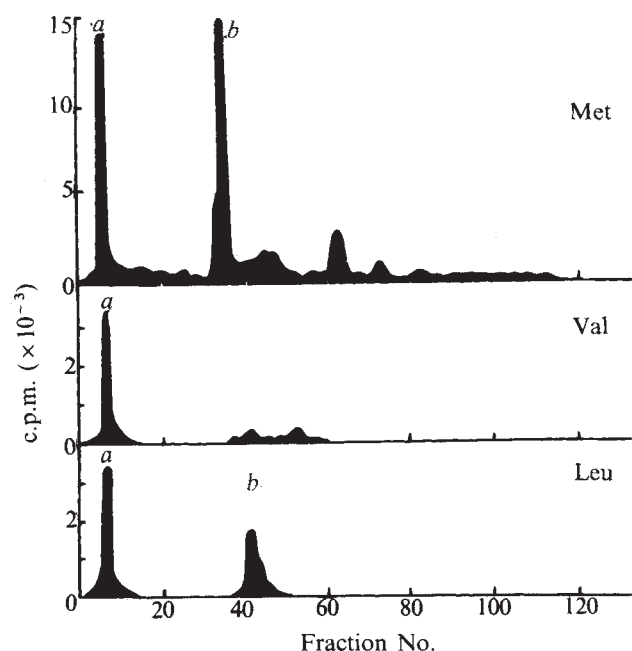


Fig. 4 Estimation of the number of cysteine peptides in tryptic digests of the enzymes. The proteins were reduced and ^{14}C -carboxymethylated in 6 M guanidine hydrochloride, digested with trypsin and the soluble peptides subjected to ion-exchange chromatography under the conditions described in Fig. 1. Aliquots of each fraction obtained from the column were counted for radioactivity and two-dimensional mapping showed that the major radioactive peaks from methionyl- and leucyl-tRNA synthetase contained one radioactive tryptic peptide each, whereas the major peak from valyl-tRNA synthetase contained two radioactive tryptic peptides.

probably a noncysteine peptide which was labelled by the rather drastic conditions used to ensure complete labelling.

It is clear from Table 4 that methionyl-, valyl- and leucyl-tRNA synthetase yield substantially fewer tryptic peptides than the expected number. In all cases the observed number is half, or less than half, the expected number. It is particularly significant that this applies to the histidine and (CM) cysteine peptides since the numbers concerned are quite small. These data, although of a negative nature, strongly suggest that the duplicated regions of these protomers are very similar.

Three lines of evidence

Our conclusion that the protomers of methionyl-, valyl- and leucyl-tRNA synthetase from *B. stearothermophilus* contain repeated sequences is based on three lines of evidence. First, each protomer contained more than one copy of several tryptic peptides. Second, widely separated

regions of the protomers contained the same tryptic peptides. Third, all three protomers yield far fewer tryptic peptides than expected if they contained unique sequences over their entire lengths. Taken together, the data also suggest that the repeating units are about half the length of the protomers and that the units within each protomer are very similar in sequence.

These models are unusual since they suggest that in each case two very similar sequences have become fused to yield the functional protomer. Proteins can acquire such repeats by incorporating partial or complete duplications of their cistrons and some proteins have evidently elongated by this process, for example, immunoglobulins¹⁵.

In most of the known cases, however, the sequences of the repeating units have subsequently diverged to an extent substantially beyond that envisaged for the aminoacyl-tRNA synthetases which seem to resemble the haptoglobin α_2 chain which is also composed of two virtually identical sequences fused through gene duplication and fusion¹⁶. It is therefore plausible that the same mechanism has generated the elongated protomers present in some aminoacyl-tRNA synthetases although the possibility of post-transcriptional or post-translational fusion cannot be excluded.

The existence of long duplicated sequences in several of the aminoacyl-tRNA synthetases raises the possibility that other proteins, especially those with relatively long protomers, have elongated in this manner. In fact, there is evidence which suggests that rabbit muscle phosphofructokinase may be one such protein¹⁷.

In none of these cases, however, is the functional advantage of such structures obvious and other information such as the folding of the 'intra-units' and the distribution of active sites between them will probably be necessary to clarify this question. One consequence of the existence of these structures, however, is that it disposes of the objection that the protomers of the aminoacyl-tRNA synthetases differ too much in size to have evolved from a common precursor, since the basic units of sequence are of comparable length although the physical lengths of the protomers are different.

We thank Dr M. F. Perutz for advice on the preparation of the manuscript. Y.B. was the recipient of a travelling fellowship from the European Molecular Biology Organisation.

Received October 22, 1973; revised February 5, 1974.

- Holley, R., Appgar, J., Everett, G., Madison, J., Marquisee, M., Merrill, S., Penswick, J., and Zamir, A., *Science*, **147**, 1462 (1965).
- Arndt, D. J., and Berg, P., *J. biol. Chem.*, **245**, 665 (1970).
- Yarus, M., and Berg, P., *J. molec. Biol.*, **28**, 479 (1967).
- Blanquet, S., Fayat, G., Waller, J. P., and Iwatsubo, M., *Eur. J. Biochem.*, **24**, 461 (1972).
- Muench, K. R., and Joseph, D. R., *Miami Winter Symposium*, **2**, 172 (1971).
- Fasolo, F., Befort, N., Boulanger, Y., and Ebel, J. P., *Biochim. biophys. Acta*, **217**, 305 (1970).
- Ostrem, D. L., and Berg, P., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1967 (1970).
- Andrews, P., *Biochem. J.*, **91**, 222 (1964).
- Laemmli, U. K., *Nature*, **227**, 680 (1970).
- Ullmann, A., Goldberg, M. E., Perrin, D., and Monod, J., *Biochemistry*, **7**, 261 (1968).
- Hartley, R. W., and Barber, E. A., *Nature new Biol.*, **235**, 15 (1972).
- Schroeder, W. A., *Methods in Enzymology*, **25**, 203 (1972).
- Crestfield, A. M., Stein, W. H., and Moore, S., *J. biol. Chem.*, **238**, 2413 (1963).
- Edelman, G. M., Cunningham, B. A., Reeke, G. N., Becker, J. W., Waxdal, M. J., and Wang, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2580 (1972).
- Black, J. A., and Dixon, G. H., *Nature*, **218**, 736 (1968).
- Coffee, C. J., Aaronson, R. P., and Frieden, C., *J. biol. Chem.*, **248**, 1381 (1973).
- Edelhoc, H., *Biochemistry*, **6**, 1948 (1967).
- Edelhoc, H., *J. biol. Chem.*, **243**, 3557 (1968).

Direct measurement of electric current generation by cytochrome oxidase, H^+ -ATPase and bacteriorhodopsin.

L. A. Drachev, A. A. Jasaitis, A. D. Kaulen A. A. Kondrashin, E. A. Liberman,
I. B. Nemecek, S. A. Ostroumov, A. Yu. Semenov and V. P. Skulachev

Department of Bioenergetics, Laboratory of Bioorganic Chemistry Moscow State University;
Institute of Problems of Information Transmission, USSR Academy of Sciences, Moscow, USSR

SUMMARY. A method for association of proteoliposomes with planar phospholipid membrane has been elaborated, by which operation of molecular electric generators, such as cytochrome oxidase, H^+ -ATPase and bacteriorhodopsin, can be followed using ordinary electrometer techniques.

The existence of molecular electric generators in mitochondrial, chloroplast and bacterial membranes was postulated by Mitchell in 1966 who proposed that enzymes of respiratory and photosynthetic redox chains and ATPases localised in these membranes catalyse transmembraneous movement of electrons or protons against the electric gradient. As a result, the membranes should charge like a condenser and electrical energy accumulated in this way might be utilised for ATP synthesis or osmotic work.

This hypothesis is difficult to verify by direct methods because of the small size of intracellular organelles and bacterial cells and the complexity of the enzymic and ion transport processes occurring in these systems. To detect the electric membrane potential in organelles and bacteria, therefore, some indirect methods were developed, such as measurement of electrophoretic transport of penetrating ions, and the electrochromic shifts of carotenoid and chlorophyll spectra²⁻⁸. In this laboratory, the direct measurement of electromotive force (e.m.f.), produced by one of the molecular electric generators, (bacteriorhodopsin) has been accomplished⁹. *Halobacterium halobium* membrane sheets containing bacteriorhodopsin as the only protein were mixed with a decane solution of phospholipids. The mixture was used to form a planar artificial membrane onto the aperture in the Teflon septum separating two electrolyte solutions. Illumination of the bacteriorhodopsin-containing membrane was found to induce generation of a transmembrane electric potential difference which was measured by a sensitive voltmeter. All attempts to use the same procedure for making planar phospholipid membrane containing cytochrome oxidase and mitochondrial proton-translocating ATPase (H^+ -ATPase) failed, however.

Direct measurement

Further experiments yielded a more universal method of direct measurement of the generation of an electric charge by membraneous proteins. The principle of this method is that, firstly, the protein is incorporated into the liposome membrane according to procedure of Racker and associates¹⁰⁻¹² which includes mixing cholate solution of a phospholipid and protein and removal of cholate by means of dialysis. Then, the resulting

proteoliposomes are brought into contact with the planar phospholipid membrane. To induce fusion of the planar and proteoliposomal membranes, the negative surface charges of phospholipids are neutralised by Ca^{2+} ions. As voltmeter measurements showed, cytochrome oxidase, H^+ -ATPase and bacteriorhodopsin, integrated with planar membrane in such a fashion, can generate a transmembrane electric potential difference at the expense of ascorbate – O_2 electron transfer, ATP hydrolysis and light energy, respectively.

Cytochrome oxidase was purified after the method of Yonetani¹³ coupling factor F_1 after Horstman and Racker¹⁴, ATPase hydrophobic proteins after Kagawa and Racker¹⁰, and bacteriorhodopsin membrane sheets after Oesterhelt and Stoekenius¹⁵. For the procedure of the cytochrome oxidase and ATPase proteoliposome preparations, see ref. 16; for bacteriorhodopsin proteoliposomes, see ref. 17.

Electric potential difference across the planar membrane was measured with Ag/AgCl electrodes connected with the RFT VA J-51 vibrating capacitor electrometer and KSP-4 recorder. Planar phospholipid membrane was made of soya bean phospholipids (azolectin) solution in decane (70 mg azolectin per ml decane). The azolectin solution was applied to a 1 mm aperture in the Teflon septum which separated the experimental vessel into two compartments containing incubation mixture. After the planar membrane was formed, proteoliposomes were added into one of the compartments. In the 15-30 min required for the proteoliposomes to become associated with the planar membrane, an energy source was introduced and the membrane potential was measured.

Proteoliposome systems

The data of a typical experiment with cytochrome oxidase proteoliposomes are given in Fig. 1. As shown in Fig. 1a, ascorbate addition to the compartment, containing cytochrome oxidase proteoliposomes and cytochrome *c*, results in the formation of an electric potential difference across the planar membrane (plus in the compartment with proteoliposomes). Cyanide reverses the effect when added after ascorbate. In the experiment shown in Fig. 1b, ascorbate (– cytochrome *o*) and proteoliposomes were added to the two different compartments. In this case, no membrane potential was formed until the mixture was supplemented with a penetrating hydrogen atom carrier, phenazine methosulphate (PMS). Direction of the field was the same as in the preceding experiment. An electric potential of opposite polarity (minus on the proteoliposome side) was generated when cytochrome oxidase proteoliposomes containing cytochrome *c* inside were used. Again, PMS was found to be necessary (not shown in Fig. 1).

In Fig. 1c the result of the experiment with ATPase proteoliposomes is demonstrated. The addition of ATP to the com-

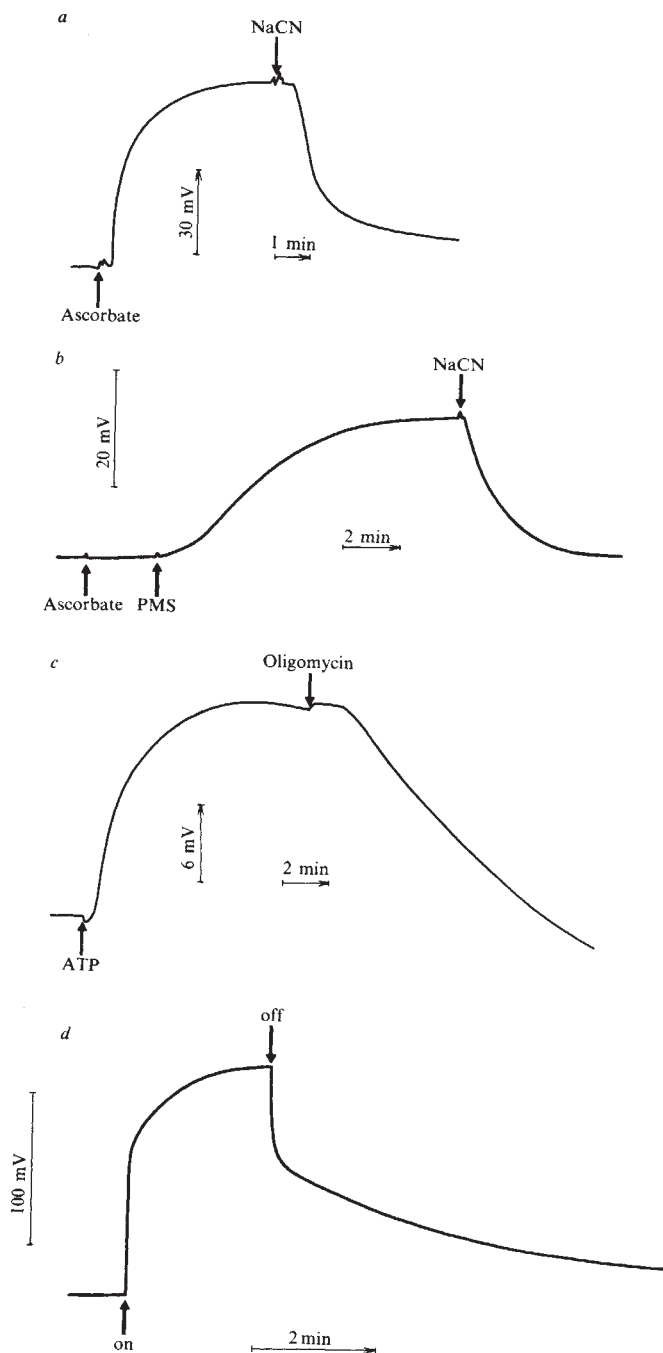


Fig. 1 Generation of transmembrane electric potential differences by cytochrome oxidase (*a*, *b*), H^+ - κ -ATPase (*c*) and bacteriorhodopsin (*d*). *a*, Incubation mixture: 0.3 M sucrose, 5 mM Tris-citrate ($pH = 7.2$), 30 mM $CaCl_2$, 5 mM $MgSO_4$, 1×10^{-4} M cytochrome *c*. One of compartments was supplemented with cytochrome oxidase proteoliposomes (0.3 mg protein ml^{-1}). Additions: 10 mM ascorbate (added in the same compartment as proteoliposomes), 1 mM NaCN (added in both compartments). Planar membrane resistance (R) 1.5×10^{10} ohm, current at maximal potential difference (I) 5×10^{-12} A. *b*, Incubation mixture: 0.2 M sucrose, 0.05 M Tris-HCl ($pH = 7.2$), 30 mM $CaCl_2$, 7×10^{-5} M cytochrome *c*. One of compartments contained cytochrome oxidase proteoliposomes (0.25 mg protein ml^{-1}). Additions in the proteoliposome-free compartment: 5 mM ascorbate, 1.5×10^{-6} M PSM, in both compartments: 1.5×10^{-3} M NaCN. $R = 1 \times 10^{10}$ ohm, $I = 2 \times 10^{-12}$ A. *c*, Incubation mixture: 0.3 M sucrose, 5 mM Tris-citrate ($pH = 7.2$), 30 mM $CaCl_2$, 5 mM $MgSO_4$. One of compartments contained ATPase proteoliposomes (0.3 mg protein ml^{-1}). Additions: 2.5 mM ATP, oligomycin (30 μg ml^{-1}). $R = 1 \times 10^{10}$ ohm, $I = 1.8 \times 10^{-12}$ A. *d*, Incubation mixture: 0.2 M sucrose, 5 mM Tris-HCl ($pH = 7.2$), 30 mM $CaCl_2$, and in one of compartments, bacteriorhodopsin proteoliposomes (0.2 mg protein ml^{-1}). $R = 2 \times 10^{10}$ ohm, $I = 7.5 \times 10^{-12}$ A.

partment with proteoliposomes gives rise to the formation of electric potential difference (minus on the proteoliposome side). Oligomycin reverses the ATP action.

Figure 1*d* illustrates the light-induced generation of an electric field by bacteriorhodopsin proteoliposomes. In this case, minus was on the proteoliposome side. In the dark, the membrane potential dissipated.

In all cases, addition of a protonophorous uncoupler, trichlorocarbonyl cyanide phenylhydrazine (2×10^{-7} M) or the shunting of the planar membrane by an external resistance greatly decreased the above responses, and accelerated their dissipation when no more energy was supplied.

The magnitudes of the membrane potential vary depending on the conditions and quality of proteoliposomes. The maximal potential values for cytochrome oxidase were ~ 110 mV, for bacteriorhodopsin ~ 150 mV. In the ATPase experiments, the membrane potentials were always lower, and more time was required for the proteoliposomes and planar membrane to fuse, the fact which may be due to the complex profile of the ATPase proteoliposome surface covered with the F_1 knobs.

Electrical response

The bacteriorhodopsin system has proved to be the most convenient experimental model, because the responses were large, steady and easily reproducible. This system was studied in some detail. In particular, the change of the photoeffect as the function of the potential difference generated across the planar membrane by an external source (ΔV_{ext}) has been measured. It was found (Fig. 2) that ΔV_{ext} of the same sign as that of the photo e.m.f. decreases, and of opposite sign increases, the light-induced response. At a certain ΔV_{ext} , the photoeffect becomes equal to zero. In these conditions, ΔV_{ext} should be equal to the bacteriorhodopsin photo e.m.f. if all bacteriorhodopsin generators have the same orientation. Photo e.m.f. should be higher than this value if some of generators are oriented in the opposite direction to the majority. According to Fig. 2, bacteriorhodopsin photo e.m.f. is ≥ 300 mV.

It seems most probable that these electrical responses are due to proteoliposomes attached to the surface of the planar phospholipid membrane. As shown previously^{18,19}, the addition of Ca^{2+} induces the fusion of two planar phospholipid membranes resulting in the formation of an electrical contact between the membranes and a local decrease in the electrical resistance.

The fusion of proteoliposomes with planar membrane seems to be irreversible. In fact, experiments with the bacteriorhodopsin system showed that the photoeffect, if it appears, is retained after a solution without proteoliposome is substituted for the proteoliposome-containing mixture.

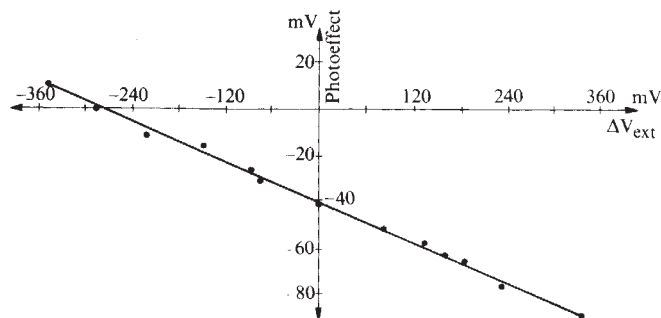


Fig. 2 Relationship between the bacteriorhodopsin-mediated photo effect and external ΔV . Incubation mixture: 0.3 M sucrose, 0.09 M $(NH_4)_2SO_4$, 5 mM Tris-HCl ($pH = 7.2$), bacteriorhodopsin proteoliposomes in one of compartments (0.1 mg protein ml^{-1}).

Proteoliposomal and planar membrane fusion

The schemes for fusion of proteoliposomal and planar membranes are given in Fig. 3. Figure 3A indicates that cytochrome oxidase proteoliposome, associated with a planar membrane, carries out electron transfer from the extraproteoliposomal ascorbate to the intraproteoliposomal oxygen.

As a result, the proteoliposome interior charges negatively. H^+ ions or other cations of the proteoliposome interior move (down the electric gradient) across the proteoliposome membrane from the same compartment (where the proteoliposomes are), or, alternatively, from the opposite compartment situated on the other side of the planar membrane (Fig. 3, dotted lines). The latter situation should give rise to the formation of an electric potential difference across the planar membrane, namely, positive charging of the proteoliposome-containing

compartment and negative charging of the opposite compartment. In agreement with this scheme, experiments on the proteoliposomes of different types showed that the proteoliposome-free compartment always charges like the proteoliposome interior. In fact, cytochrome oxidase proteoliposomes with cytochrome *c* inside, ATPase proteoliposomes and bacteriorhodopsin proteoliposomes generate positive charges, whereas the cytochrome oxidase proteoliposomes with cytochrome *c* outside generate negative charges in their interior^{16,17}. The first three types of proteoliposomes charge the proteoliposome-free compartment positively, and the last charge it negatively.

The scheme given in Fig. 3A also explains why phenazine methosulphate is required for membrane potential to be supported by oxidation of ascorbate added into the proteoliposome-free compartment. Planar phospholipid membrane is impermeable to ascorbate (shown by special experiments) so reducing equivalents cannot reach cytochrome oxidase, if proteoliposomes and ascorbate are in different compartments, until a lipid-soluble hydrogen atom carrier is added (see Fig. 1b).

An important prediction of the scheme illustrated in Fig. 3A is the inefficiency of cytochrome oxidases localised in the region of fusion of the proteoliposome with the planar membrane. According to the procedure used in the cytochrome oxidase experiments, cytochrome *c* was added several minutes later than proteoliposomes, so that the fusion preceded the reconstitution of the cytochrome *a*-cytochrome *c* complex. Moreover, ascorbate concentration should be very low in the fusion region because of hydrophilic properties of this compound. Low membrane permeability for ATP and H^+ can be responsible for ineffectiveness of ATPase and bacteriorhodopsin molecules in the fusion region, respectively.

In the reconstitution of proteoliposomes, transmembrane orientation of proteins may be of two opposite directions. To actuate the systems oriented in one of these directions, non-penetrating substrates or catalytic factors may be used: ascorbate and cytochrome *c* in the case of cytochrome oxidase proteoliposomes; ATP and factor F_1 in the case of ATPase proteoliposomes. Asymmetric reconstitution of the bacteriorhodopsin proteoliposomes (Fig. 3B) may be a result of the difference in areas of the inner and outer surfaces of the vesicles (for discussion, see ref. 17).

In Fig. 3C, the equivalent electric scheme of the experiment on estimation of the e.m.f. of the molecular electric generators is given.

We wish to thank Professor Yu. A. Ovchinnikov for advice and Professor L. P. Kayushin and Dr L. N. Chekulaveva for providing *Halobacterium halobium* cells.

This study was carried out within the framework of the Project 'Rhodopsin' organised by the USSR Academy of Sciences and Moscow State University and supervised by Professor Yu. A. Ovchinnikov.

Received January 17, 1974.

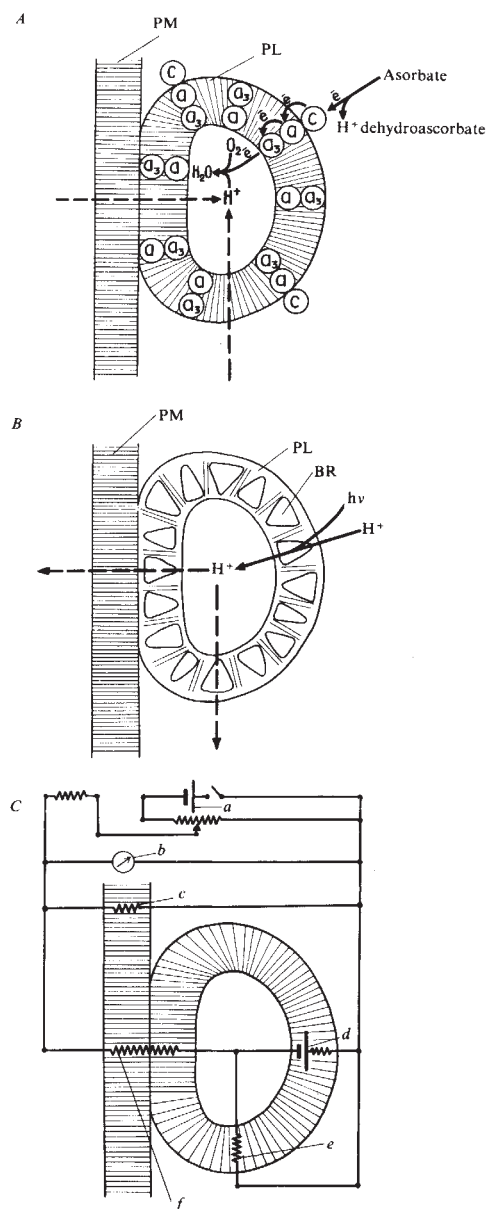


Fig. 3 Schemes illustrating interaction of the proteoliposomes (PL) with the planar membrane (PM). A, Cytochrome oxidase proteoliposomes with cytochrome *c* outside. *a*, *a*₃ and *c*—corresponding cytochromes; B, bacteriorhodopsin (BR) proteoliposomes; C, equivalent scheme for estimation of e.m.f. of the molecular electric generators. *a*, External battery; *b*, voltmeter; *c*, planar membrane resistance; *d*, molecular e.m.f. generator; *e*, proteoliposome resistance; *f*, resistance of fusion region.

¹ Mitchell, P., *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research, (Bodmin, 1966).

² Mitchell, P., *Chemiosmotic Coupling and Energy Transduction*, Glynn Research (Bodmin, 1968).

³ Greville, G. D., *Curr. Top. Bioenergetics*, **3**, 1 (1969).

⁴ Skulachev, V. P., *FEBS Lett.*, **11**, 301 (1970).

⁵ Liberman, E. A., and Skulachev, V. P., *Biochim. biophys. Acta*, **216**, 30, (1970).

⁶ Skulachev, V. P., *Curr. Top. Bioenergetics*, **4**, 127 (1971).

⁷ Witt, H. T., *J. Bioenergetics*, **3**, 47 (1972).

⁸ Skulachev, V. P., *Energy Transformation in Biomembranes* (Nauka, Moscow, 1972).

⁹ Drachev, L. A., Kaulen, A. D., Ostroumov, S. A., and Skulachev, V. P., *FEBS Lett.*, (in the press).

¹⁰ Kagawa, Y., and Racker, E., *J. biol. Chem.*, **246**, 5477 (1971).

¹¹ Racker, E., *J. Membrane Biol.*, **10**, 221 (1972).

¹² Kagawa, Y., *Biochim. biophys. Acta*, **265**, 297 (1972).

¹³ Yonetani, T., in *Methods in Enzymology* (edit. by Estabrook, R. W., and Pullman, M. E.) **10**, 332 (Academic Press, New York, 1967).

- ¹⁴ Horstman, L. L., and Racker, E., *J. biol. Chem.*, **245**, 1336 (1970).
¹⁵ Oosterhelt, D., and Stoeckenius, W., *Nature new Biol.*, **233**, 149 (1971).
¹⁶ Jasaitis, A. A., Nemecek, I. B., Severina, I. I., Skulachev, V. P., and Smirnova, S. M., *Biochim. biophys. Acta*, **275**, 485 (1972).

- ¹⁷ Kayushin, L. P., and Skulachev, V. P., *FEBS Lett.*, (in the press).
¹⁸ Liberman, E. A., and Nenashev, V. A., *Biophysika* (USSR) **13**, 193 (1968).
¹⁹ Blioch, Z. L., Glagoleva, I. M., Liberman, E. A., and Nenashev, V. A., *J. Physiol. Lond.*, **199**, 11 (1968).

Contractile units in vertebrate smooth muscle cells

J. V. Small*

Department of Zoology, Melbourne University, Parkville, 3052, Australia.

Evidence from isolated smooth muscle cells in rigor indicates that the contractile elements are grouped into separate fibrils or 'contractile units' arranged obliquely with respect to the cell axis.

FROM X-ray diffraction studies^{1,2} of living vertebrate smooth muscle, the presence of myosin filaments during both relaxation and contraction is generally accepted but the form and arrangement of these filaments within the cell is far from settled. This latter dilemma arises because the appearance of the contractile apparatus as observed in electron microscope studies of whole muscle is extremely variable³⁻¹⁵, a situation which derives mainly from the marked lability of the myosin-containing filaments under conditions of chemical fixation.

Another approach to the problem of smooth muscle structure was suggested by the demonstration¹⁶ that viable smooth muscle cells which retain their spindle-like shape may be separated from toad stomach by conventional cell separation methods, utilising collagenase and trypsin. These cells were shown to contract on electrical stimulation^{16,17}. If model cells could be produced from such preparations, corresponding to isolated myofibrils from striated muscle, whose ability to contract could be controlled through the external environment, then the contractile apparatus should be more accessible to both fine structural and biochemical studies. The same sort of approach has been particularly fruitful in investigations of nonmuscular motile systems¹⁸⁻²⁰.

With this object in mind, experiments were carried out to develop methods for the preparation of cells from smooth muscles such as chick gizzard, guinea pig taenia coli and guinea pig vas deferens which have already been the subject of several ultrastructural and biochemical investigations. The results from the first stage of this study are summarised in the present report. They show that under conditions whereby the cell membrane is made permeable, either through the cell dispersion process itself, by treatment with the nonionic detergent Triton X-100 or by glycerination, a very regular organisation of obliquely arranged birefringent fibrils is seen in the light microscope. From the ability of such cells to contract in the presence of ATP it is proposed that these fibrils represent the elemental 'contractile units' of the vertebrate smooth muscle cell.

The fibrils

Gizzards from chicks 2-3 weeks old, were thinly sliced and then diced into small pieces in Solution 1, a modified Hanks solution (NaCl, 137; KCl, 5; Na₂HPO₄, 1.1; KH₂PO₄, 0.4; NaHCO₃, 4 and glucose, 5.5 mM, respectively; Mg²⁺ and Ca²⁺ ions absent) buffered additionally with PIPES 5 mM (pH 7.1-7.4) and with added EGTA (2 mM). (The effect of EGTA was two fold: it acted first as a

relaxant so that cells could be obtained at an extended length and second as an effective pretreatment for subsequent cell dispersion by collagenase²¹.) In some experiments EGTA was replaced by hyoscine (10⁻⁶ g ml⁻¹). After 2-4 h at room temperature the muscle pieces were then transferred to 10-20 ml of a solution containing 1 mg ml⁻¹ collagenase (Worthington CLS or Sigma type I) of the same composition as above except that EGTA and phosphate were omitted and CaCl₂ (5 mM) added to activate the enzyme. With slow stirring at 37° C up to three cell collections were made at 1 h intervals, fresh reaction solution being added to the muscle pieces after each harvest. The cells were gently pelleted and washed and stored in Solution 1.

In the light microscope these preparations showed a mixture of whole cells and cell fragments (Fig. 1a). The striking feature of both of these components was that they nearly all exhibited, in phase contrast, a regular criss-cross pattern of fine fibrils within the cytoplasm (Fig. 2a). The fibrils normally averaged about 0.4 µm in width (range 0.3 - 0.5 µm) with an average separation between parallel neighbours of about 0.9 µm (range 0.8 - 1.1 µm).

Evidently, for the cell fragments, the intracellular contents were in direct contact with the bathing medium and from the following observations it was concluded that the whole cells had also been rendered permeable by the separation procedure. The intense reddish colour of gizzard which is due predominantly to an intracellular pigment and not to the vasculature supplying this tissue, is gradually lost from the muscle pieces and is absent from pellets of the isolated cells. In addition, placing the cells in solutions of widely varying osmolarities caused no change in their dimensions. These general conclusions about the permeability of gizzard cells isolated with collagenase are consistent with the recent results of Kominz and Gröschel-Stewart²².

In conditions for which the sarcolemma is disrupted and ATP and other soluble components lost from the cell, striated muscle adopts the well known 'rigor' state in which myosin crossbridges are firmly bound to actin²³. That the cells from gizzard had adopted a similar rigor-like state was confirmed by unsuccessful attempts to stimulate them with a microelectrode (experiments carried out in collaboration with Dr R. Purves). Pushing the cells along with the microelectrode tip revealed that they were extremely stiff, rigid structures.

Among the various other methods tried, glycerinated gizzard pieces (immersed in 50% glycerol, 80 mM KCl, 5mM PIPES, pH 6.5 with or without 0.5 mM dithiothreitol and stored at -20° C for 4-6 weeks) were dissociated in the same manner as described above for fresh muscle. Such experiments yielded, by comparison with fresh muscle, a rather low proportion of 'intact' cells. These cells, however, showed the same regular fibrillar pattern as illustrated in Fig. 2.

In general, the mitochondria and nuclei in the gizzard myofibrils were rather swollen and sometimes partly obscured the fibrillar organisation. These organelles could,

*On leave from the Department of Molecular Biology, Aarhus University, DK 8000, Denmark.

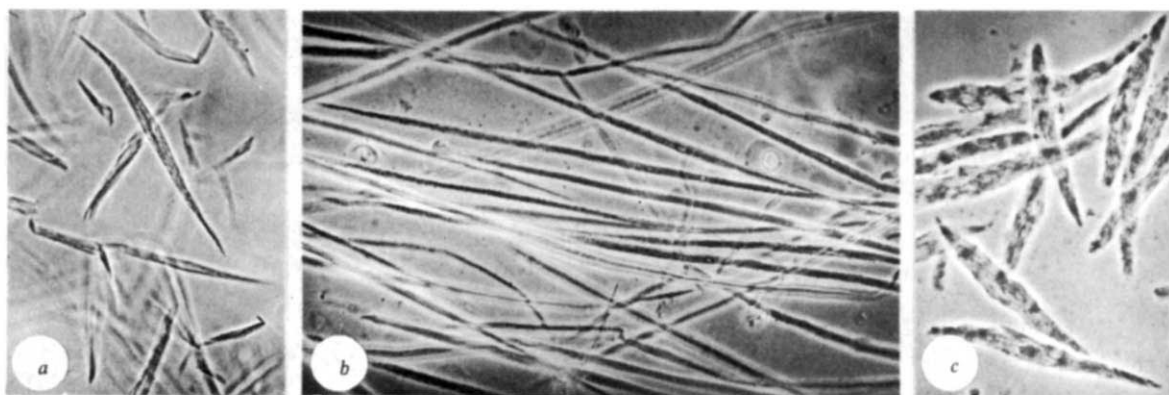


Fig. 1 Isolated cell preparations (phase contrast) *a*, Chick gizzard cells and cell fragments ($\times 165$); *b*, cells separated from extended taenia coli muscle (0.02% Triton X-100); average cell length $450\ \mu\text{m}$ ($\times 218$); *c*, cells separated from short taenia coli muscle (0.02% Triton X-100), average cell length $115\ \mu\text{m}$ ($\times 297$).

however, be removed by brief treatment of the cells with the nonionic detergent Triton X-100 (0.1% (v/v) in Solution 1) to yield 'clean' cells showing only the presence of the fine fibrils (Fig. 2*b*).

For adult guinea pig taenia coli and vas deferens, a different result was obtained when using essentially the same procedure for these muscles as employed for fresh gizzard. Collagenase treatment (carried out by necessity in the presence of Ca) caused the cells in these muscles to shorten, and, if the treatment was long enough, to round up. Such shortened cells could then be grown successfully in tissue culture indicating that, in contrast to the gizzard cells, their membranes had not been ruptured by the separation procedure. But because of their short and irregular form, these cells were unsuitable for structural studies.

Rupture of cell membranes

As one means of obtaining cells at their *in situ* length, methods were employed to deliberately rupture the cell membranes before or during collagenase treatment on the assumption that this would induce them directly into a rigor-like state, comparable to that found for gizzard. This was most readily achieved by the addition of Triton X-100 during incubation of the muscle in collagenase. Of the various concentrations tried, the most suitable were found to be 0.01–0.03% (v/v) for taenia coli and 0.1% (v/v) for vas deferens. It was also found more satisfactory to leave these muscles intact and tie them at the desired length to plastic plates during processing in collagenase. After collagenase treatment and after transfer to Solution 1, the cells could be teased from the muscle with fine

needles. A more detailed account of the various methods used will be described elsewhere.

The type of preparation obtained in this way from taenia coli muscles extended to their maximal length in the presence of EGTA is shown in Fig. 1*b*. The cells are very long, straight and spindle-shaped and, although not visible in this micrograph, their nuclei are intact. The extended length of cells from taenia coli averaged $450\ \mu\text{m}$ (77 cells—range 320 – $570\ \mu\text{m}$) with a corresponding nuclear length of between $30\ \mu\text{m}$ and $50\ \mu\text{m}$.

At relatively low magnifications with phase contrast microscopy ($\times 300$ – $\times 500$), the cell cytoplasm appears essentially homogeneous but using a $\times 100$ oil immersion objective (total magnification about $\times 1250$) a regular fibrillar pattern is clearly resolved. Observation by polarisation optics showed the fibrils to be strongly birefringent and in these conditions they are most clearly demonstrated (Figs 3*c* and 4).

The average width of the fibrils was about $0.3\ \mu\text{m}$ (range 0.26 – $0.39\ \mu\text{m}$) and the centre-to-centre separation between parallel neighbours about $0.5\ \mu\text{m}$ (range 0.45 – $0.58\ \mu\text{m}$). No abrupt discontinuities such as banding could be detected along a single fibril.

Taenia coli strips shorten spontaneously at 37°C in a normal balanced salt solution or in a similar medium such as used here for collagenase digestion. Treatment of such strips with collagenase together with Triton X-100 as above yields shortened cells (Fig. 1*c*). The average length of these shortened cells was about $115\ \mu\text{m}$ (100 cells—range 65 – $160\ \mu\text{m}$). In these cells the fine fibrils were seen to be considerably more oblique (Fig. 3, *a* and *b* forming angles up to about 24° (at about $115\ \mu\text{m}$) with respect to the

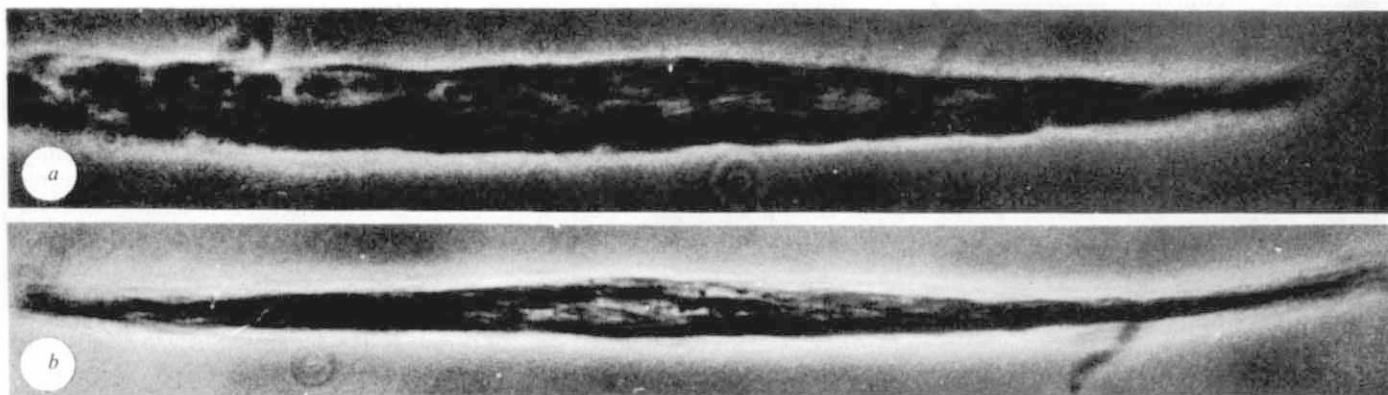


Fig. 2 Cells from chick gizzard (Phase contrast) *a*, cell at high magnification showing obliquely oriented fibrils within the cytoplasm ($\times 1246$); *b*, gizzard cell from a preparation treated additionally with Triton X-100 (see text), again showing the fibrils ($\times 774$).

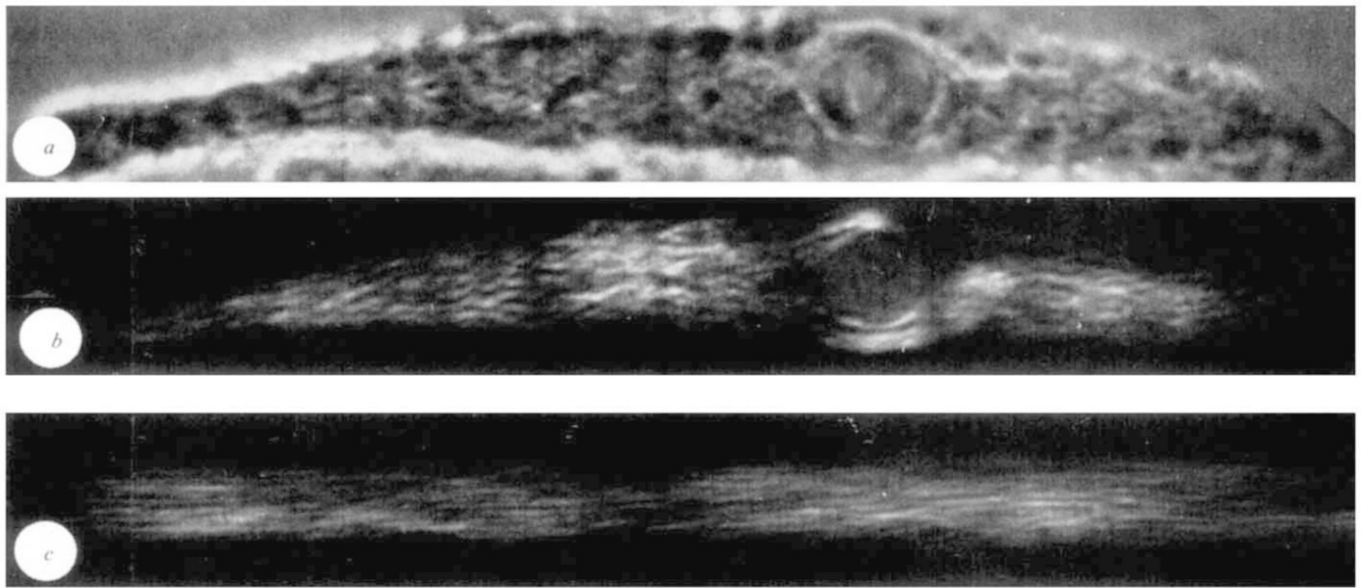


Fig. 3b and **c**, short cell from taenia coli (from a preparation such as shown in Fig. 1c). The cell length is about 90 μm . The fibrils are considerably more oblique with respect to the cell axis as compared to the extended cells (Figs 3a and Fig. 4). Polarisation optics and phase contrast respectively ($\times 1970$). **c**, Part of an extended taenia coil cell (from a preparation such as shown in Fig. 1b) observed with polarisation optics. The fibrils are birefringent and subtend angles up to about 5° with respect to cell axis ($\times 2340$).

cell axis, as compared to about 5° in the extended cells (Fig. 3c). Cells could also be found which had contracted to as short as about 35 μm . These supercontracted cells had an irregular outline and their fibrils subtended angles up to 40° with respect to the cell axis. Cells from the vas deferens contained fibrils of similar dimensions to those observed in the taenia coli cells.

By comparison with the taenia coli and vas deferens, the fibrillar pattern in the gizzard cells was as already indicated, normally rather coarse and could be recognised at quite low optical magnifications. It was, however, not uncommon to find cells with a much finer fibrillar lattice with fibrils having a similar width to those observed in the taenia coli muscle. Some variation in fibril width was also recognised, but to a lesser extent, in cells from the taenia coli and vas deferens. The reason for these differences is at present unknown, but indicates that some subdivision or association of fibrils can occur.

Response to ATP

Efforts to cause gizzard and taenia coli cells to contract showed that they were capable of shortening in the presence of ATP. Cells suspended under a coverslip in 80 mM KCl, 4 mM MgCl_2 , 5 mM PIPES, pH 6.5–6.8, could be induced to contract by the addition of 0.1–4 mM ATP in the same buffer to the coverslip edge.

The cells reacted to ATP by slowly shortening over a variable time period, but which was at the shortest in the order of about 1 min. Cells from taenia coli shortened to an average length of about 65 μm (40 cells, range 40–100 μm).

The changes in appearance of the cells that accompanied contraction as seen under phase contrast conditions were slightly variable and for taenia coli, two different responses could be recognised. In one case the initiation of contraction was accompanied by a roughening of the cell, apparently on its surface. Although this roughening effect tended to mask the internal details of the cell it seemed that there was also an associated change in the fibrils such that they could no longer be recognised, a situation which persisted throughout contraction.

In the second case, ATP caused a slight swelling of the

cells, followed by slow shortening. In this situation the fibrils were seen to disperse and the cells became rather transparent. Roughening of the cell outline occurred as the cells approached their shortest length. For the gizzard preparations, the response was of the latter type described for taenia coli. After shortening had ceased, cells could then be found in which fibrils were again clearly visible and which then showed a considerable obliqueness with respect to the cell axis (that is, as in Fig. 3a and b).

In some cases, particularly with gizzard, the addition of ATP did not always produce contraction. With several preparations ATP addition caused some, or all, of the cells to swell up or to completely dissolve. Possible reasons for this variable response are the subject of further investigations. Of additional interest was that the inclusion of EGTA (2 mM) in the ATP-testing medium to chelate any contaminating calcium did not affect contraction in any way. This suggested that the Ca^{2+} regulatory mechanism of vertebrate smooth muscle^{24,25} (R. D. Bremel and A. Sobieszek, M. K. Reedy, unpublished), had been lost or rendered ineffective during the cell preparation procedure.

Relaxed cells

By the use of ATP (0.5 mM) instead of Triton in the collagenase medium, extended cells were obtained from taenia coli which were similar in general appearance to the cells obtained from toad stomach by Bagby *et al.*¹⁶ and Fay and Delise¹⁷, being smoothly curved along most of their length. From their observed movement on a slide in a stream of bathing medium, these cells were seen to be very flexible, in contrast to their rigid counterparts obtained with Triton. Furthermore, they were found to shorten immediately on transfer from the EGTA-containing medium (Solution 1) to one in which EGTA was replaced by Ca^{2+} . This shortening could, alternatively, be observed under the microscope by the addition of the medium containing Ca^{2+} to the coverslip edge.

From these findings it was apparent that the cells obtained with ATP were essentially intact and could be considered, in the absence of Ca^{2+} , to be in a 'relaxed' state. (The effects of ATP and calcium in this instance will be considered in detail elsewhere).

Under phase contrast or with polarisation optics, fibrils could not be recognised in these relaxed cells, neither could they be observed under phase contrast during contraction with Ca^{2+} .

The contractile apparatus

The present results represent the first demonstration in the light microscope of a regular fibrillar organisation within the vertebrate smooth muscle cell. Fibrils of similar dimension were often described in the early literature on smooth muscle²⁶⁻²⁸ but were not seen to form any regular pattern within the cell. It is not unlikely that what the early histologists observed was a distorted arrangement of the fibrils described here.

From the ability of the cells to contract with ATP it is clear that the presence of the fibrils is not artifactual but represents a significant state of the contractile apparatus. As already indicated this state may be considered as essentially equivalent to rigor in striated muscle. In a very recent study, Kominz and Groschel-Stewart²⁹, using a similar technique, obtained isolated cells from gizzard which, by the use of an 'electric-sizing technique' were also shown to contract with ATP. But these authors did not report the presence of fibrils within the cells.

The most reasonable interpretation of the fibrillar pattern is that it reflects directly the gross organisation of the contractile apparatus. Thus, the contractile elements (the thick and thin filaments etc) are considered as being grouped into fibrillar units, that is the fibrils, within the cell. Furthermore, as there was no evidence for interconnections between the fibrils, it is most likely that each fibril represents a separate contractile unit, presumably attached at either end to the cell surface. These units are arranged obliquely across the cell and contraction is achieved by a shortening of the fibrils, which themselves, as a result of shortening, become progressively more angled with respect to the cell axis. Such changes in fibril orientation are fully consistent with the known decrease in birefringence of smooth muscle on shortening²⁹ and with the noted disorientation of myofilaments in the shortened cell¹⁷.

The changes that occur when cells pass from rigor to the contracting state and which lead to a loss in visibility of the fibrils, clearly involve changes within the fibrils themselves. Myofibrils obtained from glycerinated striated muscle tend to swell when passing from rigor to the relaxed state³⁰. A similar swelling response to ATP would cause the fibrils here to become both less refractile as well as closer neigh-

bours, changes which could render them undetectable by light microscopy. From the results on 'relaxed cells' it is presumed that rather smaller changes occur in fibril dimensions between relaxation and contraction.

I suggest that the results presented here represent a significant step towards elucidating the organisation of the contractile apparatus of vertebrate smooth muscle. There is now strong evidence for an ordered grouping of the contractile elements into separate contractile units. On this basis, the problem of the mode of contraction in this muscle type reduces, in the first instance, to that for an individual fibril. Continuing studies are being aimed at further defining the functional significance of the fibrils and their detailed structural organisation.

I thank Professor G. Burnstock for providing the facilities for this research and thank him and Drs G. Campbell and A. Sobieszek for discussion and criticism of the manuscript. I thank Miss J. McConnell for assistance with tissue cultures, Mr B. Pump for photography and Mrs G. Liddell for typing. The author acknowledges the generous support of the Danish Science Research Council and the Zoology Department for travelling expenses. This work was supported in part by funds from the National Heart Foundation of Australia and the Australian Research Grants Committee.

Received October 1, 1973; revised January 10, 1974.

Note added in proof: By Triton treatment, in the presence of dithiothreitol, of 'relaxed' cells from taenia coli (produced with ATP) cells may be obtained which exhibit the same fibrillar organisation as cells rupture during collagenase treatment and which, further, show sensitivity to Ca^{2+} ion for contraction.



Fig. 4 A group of extended taenia coli cells (as in Fig. 3a) showing the fibrils. Polarisation optics ($\times 1162$).

- ¹ Lowy, Poulsen, F. R., and Vibert, P. P. *Nature*, **225**, 1053-1054 (1970).
- ² Lowy, J., Vibert, P. J., Haselgrove, J. C., and Poulsen, F. R., *Phil. Trans. R. Soc. B*, **265**, 191-196 (1973).
- ³ Cooke, P. H., and Fay, F. S., *J. Cell Biol.*, **52**, 105-116 (1972).
- ⁴ Devine, C. E., and Somlyo, A. P., *J. Cell Biol.*, **49**, 636-649 (1971).
- ⁵ Garamvölgyi, N., Vizi, E. S., and Knoll, J., *J. ultrastruct. Res.*, **34**, 135-143 (1971).
- ⁶ Heumann, H.-G., *Cytobiologie*, **3**, 259-270 (1971).
- ⁷ Kelly, R. E., and Rice, R. V., *J. Cell Biol.*, **42**, 683-691 (1969).
- ⁸ Lowy, J., and Small, J. V., *Nature*, **227**, 46-51 (1970).
- ⁹ Pease, D. C., *J. ultrastruct. Res.*, **23**, 280-303 (1968).
- ¹⁰ Rice, R. V., Moses, J. A., McManus, G. M., Brady, A. C., and Blasik, L. M., *J. Cell Biol.*, **47**, 183-196 (1970).
- ¹¹ Panner, B. J., and Honig, C. R., *J. Cell Biol.*, **35**, 303-321 (1967).
- ¹² Shoenberg, C. F., *Phil. Trans. R. Soc.*, **B265**, 197-201 (1973).
- ¹³ Small, J. V., and Squire, J. M., *J. molec. Biol.*, **67**, 117-149 (1972).
- ¹⁴ Somlyo, A. P., Somlyo, A. V., Devine, C. E., and Rice, R. V., *Nature new Biol.*, **231**, 243-246 (1971).
- ¹⁵ Uehara, Y., Campbell, G. R., and Burnstock, G., *J. Cell Biol.*, **50**, 484-497 (1971).
- ¹⁶ Bagby, R. M., Young, A. M., Dotson, R. S., Fisher, B. A., and McKinnon, K., *Nature*, **234**, 351-352 (1971).
- ¹⁷ Fay, F. S., and Delise, C. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 641-645 (1973).
- ¹⁸ Gibbons, B. H., and Gibbons, J. R., *J. Cell Biol.*, **54**, 75-97.
- ¹⁹ Mooseker, M. S., and Tilney, L. G., *J. Cell Biol.*, **56**, 13-26 (1973).
- ²⁰ Naitoh, Y., and Kaneko, H., *Science*, **176**, 523-524 (1972).
- ²¹ Seglen, P. O., *Expl Cell Res.*, **74**, 450-454 (1972).
- ²² Kominz, D. R., and Groschel-Stewart, U., *J. Mechanochemistry Cell Motility*, **2** (in the press).
- ²³ Huxley, H. E., in *The Cell* (edit. by Brachet, J., and Mirsky, A. E., IV, p. 365, (1960) Academic Press, New York and London.
- ²⁴ Ebashi, S., Iwakura, H., Nakajima, H., Nakamura, R., and Ooi, Y., *Biochem. Z.*, **345**, 201-211 (1966).
- ²⁵ Sparrow, M. P., Maxwell, L. C., Ruegg, J. C., and Bohr, D. F., *Am. J. Physiol.*, **219**, 1366-1372 (1970).
- ²⁶ Heidenhain, M., *Ergebn. Anat. EntwGesch.*, **10**, 115 (1901).
- ²⁷ McGill, C., *Am. J. Anat.*, **9**, 493-545 (1909).
- ²⁸ Bloom, W. E., and Fawcett, D. W., *A Textbook of Histology*, 9th edition, 264 (Saunders, Philadelphia, 19xx).
- ²⁹ Fischer, E., *J. cell. comp. Physiol.*, **23**, 113-130 (1944).
- ³⁰ Bendall, J. R., *Muscles, Molecules and Movement*, 43 (Heinemann, London, 1969).

LETTERS TO NATURE

PHYSICAL SCIENCES

Relative-distance Machian theories

MACH's principle, in essence, requires that the dynamical law of the Universe be expressed ultimately in terms only of the relative distances between the observable entities in the universe. Here I propose a general framework for constructing theories that satisfy this postulate automatically. A simple model shows how the Newtonian world picture can be satisfactorily 'Machianised.' In principle, one could attempt to Machianise the post-1905 world picture along similar lines.

The key Kinematic concept is the relative configuration space (RCS) of the Universe. If, as in Newtonian theory, the Universe is assumed to consist of N particles in a fixed three-dimensional Euclidean space, the points of the RCS (the Newtonian RCS) are all the distinct relative configurations of these N particles. Many other RCSs are conceivable. For example, the points of an RCS could be all distinct three-geometries, and the RCS would then be the 'superspace' familiar in general relativity¹. The general framework is not, therefore, tied to pre-1905 physics.

A kinematical history of the Universe (or RCS curve) is any continuous curve in the RCS. On any such curve, each point defines an 'instant of time' in the given history; the unfolding of time is but the fact of the universe's moving along some RCS curve (Leibniz's concept of time). Note that time — and therefore the possibility of defining relative velocities — is derived from within the RCS; note also the emphasis that is placed on the motion of the Universe as a whole.

If the dynamical law of the Universe is expressed solely in terms of elements taken from within the RCS, the theory must be Machian by construction. This is the general criterion that a Machian dynamics must satisfy. One possibility of realising such a dynamics is to seek a variational principle that contains only allowed kinematical elements and makes actually realised RCS curves extremal compared with neighbouring kinematically allowed curves. The initial conditions are specified by the direction of the RCS curve at a given point of the RCS. Boundary conditions do not arise.

In the case of the Newtonian RCS, space will play a geometrical but not dynamical role (in contrast to Newtonian mechanics). In a theory in which three-geometries constitute the points of the RCS, a Machian dynamics would automatically entail a variable three-geometry that unfolds in accordance with a Machian law. In this case, it would be more appropriate to speak of a relative-configuration (rather than relative-distance) Machian theory. Such an approach is not identical to the superspace approach to general relativity, in which it is assumed *a priori* that a sequence of three-geometries 'stacks' into a four-dimensional Riemannian space¹. I see here the extraneous element (not taken from within the RCS) responsible for general relativity's failure to give an entirely satisfactory account of inertia.

It seems difficult to reconcile the universal time defined here with the local time of special relativity. But the flat-space approach to general relativity^{2,3} has shown how the *a priori* assumed kinematical elements of a theory can, in fact, be rendered inobservable when a dynamical law of gravitation is imposed on a prescribed kinematical structure. In principle,

something similar could happen here — a dynamical law, perhaps in conjunction with a particular class of plausible initial conditions, could lead to a dynamics in which only a local time can in fact be observed. The present approach reverses the priorities that Einstein had in mind when constructing general relativity. Instead of requiring special relativity to hold in the small under all conceivable circumstances in all possible universes (and hoping that this can somehow be reconciled with Mach's principle *via* the equivalence principle), the aim here is to construct theories that are Machian of necessity and then to see if any of these give rise to special relativity in the small in universes like ours.

In the case of a Newtonian RCS with N point particles $i = 1, \dots, N$ of mass m_i (an intrinsic number associated with each particle; $\sum m_i = M$), the obvious allowed kinematical elements are the relative distances $r_{ij}(\lambda)$ between all pairs i and j of particles, and their derivatives $\dot{r}_{ij} = dr_{ij}/d\lambda$ with respect to an arbitrary time parameter λ along the RCS curve. The simplest nontrivial gravito-inertial dynamics is then defined by the Lagrange function

$$L = \psi \Gamma \quad (1)$$

where

$$\Gamma = \left(\sum_{i < j} m_i m_j r_{ij}^2 \right)^{1/2}, \quad i = 1, \dots, N$$

$$\psi = \sum_{i < j} m_i m_j / r_{ij}$$

Equation (1) has a product form to ensure that $L d\lambda$ is independent of the time parameter (a sum like $(\psi + \Gamma)d\lambda$ would not be λ -independent).

The equations of motion deduced from (1) in three dimensions are too complicated to be readily comprehensible. Simple equations are obtained in one dimension by introducing Cartesian coordinates, whose origin may execute any suitably continuous motion whatsoever relative to the particles in their one-dimensional universe. The resulting Euler-Lagrange equations will describe the uniquely determined relative motion (which is all that is observable) in terms of the arbitrarily chosen coordinate system. Let $x_i(\lambda)$ be the coordinate of particle i ; then L becomes

$$L = \psi \left[\sum_{i < j} m_i m_j (x_i^2 - 2x_i x_j + x_j^2) \right]^{1/2} \quad (2)$$

and the Euler-Lagrange equations

$$\frac{d}{d\lambda} \left(\partial L / \partial \dot{x}_i \right) = \partial L / \partial x_i$$

are

$$\frac{d}{d\lambda} \left\{ \frac{\psi m_i}{\Gamma} \left[\sum_{j \neq i} m_j (x_i - x_j) \right] \right\} = \frac{\Gamma \partial \psi}{\partial x_i}$$

$$\frac{d}{d\lambda} \left\{ \frac{\psi m_i}{\Gamma} \left[x_i (M - m_i) - \sum_{j \neq i} m_j x_j \right] \right\} = \frac{\Gamma \partial \psi}{\partial x_i}$$

We can now specialise both λ , taking

$$d\lambda = ds = \left(\sum_{i < j} m_i m_j dr_{ij}^2 \right)^{1/2}, \quad \text{i.e. } \Gamma \equiv 1,$$

and the coordinate system, taking it such that

$$\sum m_i x_i = 0 \quad (\text{here } d\lambda = ds)$$

In these special frames, which are distinguished by the uniquely determined relative motion, the equations of motion simplify to

$$M \frac{d}{ds} (\psi m_i x_i) = \frac{\partial \psi}{\partial x_i} \quad (3)$$

If only a few particles are present in the Universe, equations (3) clearly lead to motion that is very different from the Newtonian. On the other hand, in an environment broadly similar to ours (very many stars distributed uniformly over a large region), ψ

will be effectively constant and s will be indistinguishable from Newtonian time. Then equation (3) becomes

$$m_i d\dot{x}_i/dt = (1/M\psi) \partial\psi/\partial\dot{x}_i \quad (4)$$

where $\gamma = 1/M\psi$ is a 'gravitational constant' that is determined by the actual distribution of matter in the Universe. A different model (in preparation) turns out to have more interesting properties, so I shall not attempt to estimate γ here. In the neighbourhood of the Sun, say, the motion predicted by equation (4) is essentially indistinguishable from the Newtonian if γ has the correct value.

This model is, I believe, interesting for several reasons. First, it explains inertia (resistance of a body to rectilinear acceleration relative to the remaining bodies in the Universe) solely in terms of relative distances and relative velocities and demonstrates that a complete dynamics can be expressed in such terms. For the discussion of how Mach's principle should be implemented, this may be important, for Einstein, in particular, doubted whether the Newtonian world picture could be Machianised in this manner⁴. It is particularly satisfying that preferred frames (the inertial frames of Newtonian theory) are distinguished by a motion whose dynamics is expressible solely in terms of relative distances.

Second, although two features of Newtonian gravitation are introduced *a priori* in equation (1) — all gravitational 'charges' are of the same sign and are proportional to the inertial 'masses' — the theory itself determines the actual strength of gravity (because a coupling constant cannot enter the product L) which must be attractive. (In the model in preparation, gravity and inertia are truly subsumed into a single mechanism and the correct order of magnitude of the gravitational constant is predicted.)

Third, whereas Newtonian theory and general relativity provide equations of motion for essentially inobservable quantities in the limit of universes containing only a few particles, no such problem arises in the present approach. If there is just one particle in the Universe, the RCS does not even exist, so there is no theory at all. If there are two particles, the RCS is the positive half axis, but no equation of motion can be formulated because equation (1) becomes trivial; the particles may approach or recede but no meaning can be attached to the rate at which this happens. Nontrivial dynamics first becomes possible when there are three particles, and even then the motion is quite unlike the Newtonian. Many particles in an isotropic background are required to build up the inertial forces as we know them in this universe.

Other forces could be included readily by adding a term linear in the r_{ij} s to L (gyroscopic-type forces) or multiplying L by another factor $\Phi = \Phi(r_{ij})$. Indeed, in an attempt to include electrostatics, take $L' = \Phi\Psi\Gamma$, where $\Phi = \sum_{i < j} e_i e_j / r_{ij}$,

with $e_i = 1$ for $i = 1, \dots, N/2$ and $e_i = -1$ for $i = N/2 + 1, \dots, N$. Then the equations of motion corresponding to the conditions under which equation (4) hold are

$$m_i d\dot{x}_i/dt = (1/M\Psi) \partial\Psi/\partial\dot{x}_i + (1/M\Phi) \partial\Phi/\partial\dot{x}_i \quad (5)$$

As L' is a product, the relative strength of the new forces is again determined by the theory for each particular universe. In fact, if the particles in this case have equal masses and are distributed more or less uniformly, then $|\Psi/\Phi| \sim N$, because all the $\sim N^2$ terms in Ψ are positive, whereas there are just N more negative than positive terms in Φ . Thus, the electrostatic forces in equation (5) are $\sim N$ times stronger than the gravitational forces and like charges repel and opposites attract (provided $\Phi < 0$; I do not know whether distributions could exist for which $\Phi > 0$). As it happens there are $N \sim 10^{80}$ baryons in the observable Universe⁵, whereas we require a strength ratio $\sim 10^{40}$, but the fact that we get the square of the right answer is perhaps an indication that product Lagrangians, which arise naturally (if not uniquely) in such a global approach to motion, could perhaps provide an explanation for the famous cosmic coincidences⁵.

If the general framework is in fact the right approach to the problem of inertia, radical changes in theoretical physics are

almost certainly necessary, and it is unlikely that the simple model presented here could bear any more resemblance to an ultimately successful theory than Bohr's original model of the hydrogen atom does to modern quantum field theory.

I thank D. Sciama, C. Misner, K. Kuchar, D. Raine, and R. Breuer for discussions and encouragement and the referee for helpful comments.

J. B. BARBOUR

College Farm,
South Newington,
Banbury, Oxfordshire, UK

Received August 3, 1973; final version received February 28, 1974.

¹ Wheeler, J. A., in: *Gravitation and Relativity* (edit. by Chiu, H. - Y. and Hoffmann, W. F.), (Benjamin, New York, 1966).

² Thirring, W. *Ann. Phys.*, **16**, 96 (1961).

³ Mittelstaedt, P., and Barbour, J. B., *Z. Phys.*, **203**, 82 (1967).

⁴ Einstein, A., *The Meaning of Relativity*, chapter 3 (Methuen, London, 1922).

⁵ Bondi, H., *Cosmology* (Cambridge University Press, 1960).

Upper mass limit for quasar

THE radio source 4C11.50=1548 + 115 is reported to be a pair of quasars only 5 arcs apart^{1,2}. One quasar, 4C11.50a, has a redshift $z = 0.4359$ and is approximately 17 mag, the second quasar, 4C11.50b has a redshift $z = 1.901$ and is an object of 19 mag. I wish to point out that if the redshifts are cosmological the mass of 4C11.50a must be less than about $4 \times 10^{12} M_{\odot}$.

The light ray from the more distant b passes close to a. The impact parameter for the light ray depends somewhat on the cosmological model but may be taken as about 40 kpc ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$). If a quasar has a mass as large as a giant galaxy then the gravitational deflection of the light ray may be noticeable. Gravitational imaging has been discussed at length by many authors but the discussion presented by Press and Gunn³ is entirely adequate for this consideration. For simplicity, it can be assumed that both quasars are point masses and point light sources. When a light ray from a distant quasar b passes close to the quasar a then two images of b may be seen located on opposite sides of a. When a, b and an observer are exactly aligned, then b is seen as a ring with quasar a located at its centre. The angular radius of the ring, ϕ_0 , is of the same order of magnitude as the deflection angle of the light ray. Therefore, there is an approximate relationship: $\phi_0 \approx 2'' (M_a/M_{\odot})/(l/R_{\odot}) \approx 5 \times 10^{-8}'' (M_a/M_{\odot})/(l/\text{pc})$, where M_a is the mass of the quasar a and l is the impact parameter. If a, b and the observer are not exactly aligned, then the observer sees two images of b. If the angular separations from a of the brighter and the fainter image are ϕ_1 and ϕ_2 respectively, then

$$\phi_1 > \phi_0 > \phi_2$$

Combining the two relationships provide an estimate of the mass of a:

$$M_a/M_{\odot} < (\phi_1/5 \times 10^{-8}''/(l/\text{pc}))$$

The observed image of 4C11.50b is almost certainly the brighter, as there is no other object visible within about 1 arc min on the opposite side of 4C11.50a (ref. 2). Inserting $\phi_1 = 5''$, $l = 4 \times 10^4 \text{ pc}$ into the latter formula gives an upper mass limit for the quasar 4C11.50a:

$$M_a < 4 \times 10^{12} M_{\odot}$$

This mass limit is not excessively large. Combining the observed luminosities for cluster galaxies of the first rank⁴ with the luminosity-to-mass ratio for ellipticals⁵ gives masses of $10^{13} M_{\odot}$.

If it were possible to detect the second image of 4C11.50b the mass of 4C11.50a could be calculated. For the point-mass scatterer there is a simple relationship between the luminosity ratio and the separation ratio of the two images³. If the observed ratios did not agree with those predicted for the point

masses then information could be obtained about the mass distribution around the quasar 4C11.50a. If quasars are active nuclei of giant galaxies⁶ the existing observations indicate that the total mass within 40 kpc of the nucleus of 4C11.50a is less than about $4 \times 10^{12} M_{\odot}$. There is a weak emission in 4C11.50a at the same wavelength as a strong CIV emission in 4C11.50b (ref. 1). It may be because of the unresolved second image of 4C11.50b.

I thank Professor W. Zonn for informing me about the discovery of a close pair of quasars.

B. PACZYNSKI

*Institute of Astronomy,
Polish Academy of Sciences,
00-478 Warszawa,
Al. Ujazdowskie 4, Poland*

Received January 28, 1974.

¹ Wampler, E. J., Baldwin, J. A., Burke, W. L., Robinson, L. B., and Hazard, C., *Nature*, **246**, 203 (1973).

² Hazard, C., Jauncey, D. L., Sargent, W. L. W., Baldwin, J. A., and Wampler, E. J., *Nature*, **246**, 207 (1973).

³ Press, W. H., and Gunn, J. E., *Astrophys. J.*, **185**, 397 (1973).

⁴ Sandage, A., *Astrophys. J.*, **178**, 1 (1972).

⁵ Page, T., *Astrophys. J.*, **136**, 685 (1962).

⁶ Kristian, J., *Astrophys. J. Lett.*, **179**, L61 (1973).

Transit of Mercury across the solar disk

THE transit of Mercury across the solar disk on May 9, 1970 (4 h 20 min 30 s – 11 h 12 min 0 s UT), was observed by recording the radio emission at a wavelength of 8 mm using a 22-m paraboloid radio telescope¹. The pencil beam of the antenna is of width $D=1'6$ and the sensitivity of the radiometer ≈ 1.5 K. The antenna was steered so that Mercury was in the centre of the beam. Mercury obscures the inhomogeneities of radio emission when passing across the solar disk and some fluctuations of the flux of radio emission can occur. The characteristic time of these fluctuations and their amplitude characterise the sizes and temperatures of condensations respectively. In Fig. 1 we show a sample of original (averaged over 15 s) records of fluctuations during the passage of Mercury across the relatively active regions on the disk. Similar records, but with slightly slower fluctuations of smaller amplitude have been obtained for quiet regions of the disk. The autocorrelation curves for active (A) and quiet (Q) regions (Fig. 2) reveal details of the nature of the emission.

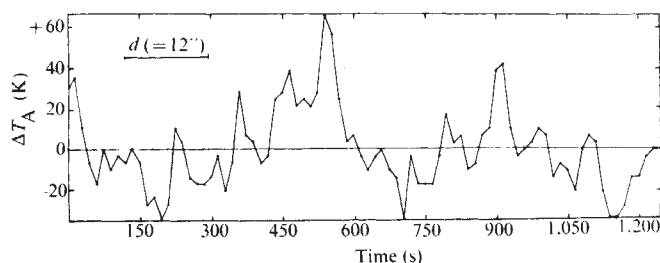


Fig. 1 Fluctuations of radio noise observed as Mercury transits the Sun.

For Q regions the characteristic sizes corresponding to maxima are $3''.4$ for the lag $m=0$ and $32''.2$ and $41''.3$ for the lags $m=44$ and $m=53$. We note that the characteristic size l of condensations is approximately determined from the relations: $l = V\tau_m/2$, if $d > 1$, and $l = V\tau_m - d$ when $d < 1$, where $\tau_m = m\Delta t$, ($\Delta t = 15$ s), v is the velocity of the transit of Mercury across the disk ($= 0''.067$ s⁻¹) and d is the size of Mercury ($12''$).

(We assume, of course, that the size of condensations of the order of the spacing between them).

For A regions the corresponding maxima appear at lags $m=0, 5, 29$, and 36 which correspond to the sizes $1''.8, 2''.51, 17''.4$, and $24''.2$, respectively. Condensations in A regions are a little more compact than in Q regions. The smallest condensations in both regions have a size of $2''$ to $3''$ which coincides with the size of smallest 'elements' of the general magnetic field². The group of larger condensations (from $17''$ to $41''$), although in the size range characteristic of larger 'elements' of general magnetic fields, reflects, we believe, the characteristic sizes of chromospheric supergranules (10^4 to 3×10^4 km²).

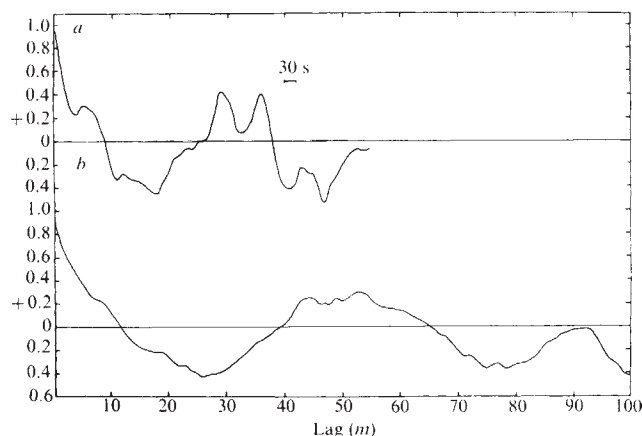


Fig. 2 Autocorrelation curves for: a, A region; b, Q region.

The characteristic amplitude of fluctuations or excess of brightness temperature ΔT_B over the background (temperature $T_{B0} = 8,000$ K for $\lambda = 8$ mm) determined as the r.m.s. of all fluctuations (deviations from the mean) depends on the ratio d/l and the value of D , and also on the character of eclipse ('central' or 'partial') of condensations by Mercury. The corresponding approximate formulae for excess of antenna temperature ΔT_A are $\Delta T_A \approx (d^2/D^2) \Delta T_B$ when $d < l$ and

$$\Delta T_A \approx 2(d/l)/(D/l)^2 \Delta T_B$$

when $d > l$. The brightness temperature excess ΔT_B for small condensations ($l < 3''$) is $\approx 5,000$ K in Q regions and 8,950 and 6,420 K in two observed A regions. For large condensations connected with supergranules the corresponding temperatures ΔT_B are 2,830 K for Q regions and 2,690 for A regions. As the background temperature is 8,000 K the true brightness temperature $8,000 + \Delta T_B$ is 13,000 K for small condensations (Q regions) and is 14,000–17,000 K in A regions. The values are characteristic of upper chromospheric temperatures and transition layer temperatures. For supergranular structures the true T_B is about 10,700 – 10,800 K in A and Q regions, typical of the lower chromosphere. In other words small condensations, which are more compact in A regions, are appreciably hotter than supergranular structures; the latter are practically of the same temperature in both regions. Condensations of radio emission do not form a continuous sequence in the regions examined and all are concentrated around characteristic size of granules and supergranules.

Could the effect observed be due to the time variations of radio emission from the whole area D . To exclude this possibility scans along the same path were repeated without Mercury. These records did not show appreciable fluctuations similar to that of Fig. 1. Moreover we have examined the cross correlation of these fluctuations with those without Mercury; the cross-correlation coefficient never reached 0.05. The fluctuations we consider here cannot therefore, be

connected with possible time variations of the radio emission of the Sun as a whole⁴.

V. A. EFANOV
I. G. MOISEEV
A. B. SEVERNY

Crimean Astrophysical Observatory,
PO Nauchny Crimea,
334413, USSR

Received December 31, 1973.

¹ Efanov, V., Moiseev, I., and Severny, A., *Int. astr. Union Symp.* No. 35 (edit. by Kiepenheuer, K. O.), 588 (1968).

² Severny, A., *Q. Jl R. astr. Soc.*, **12**, 363 (1971).

³ Leighton, R., Noyes, R., and Simon, G., *Astrophys. J.*, **135**, 471 (1962).

⁴ Durasova, M., Kobrin, M., Losovsky, J., Chandajev, A., and Judin, O., *Astr. Tsirk. Byuro astr. Soobshch.*, **531**, (1969)

Ionisation ledges in the equatorial ionosphere

A cusp formation on topside ionograms recorded at equatorial latitudes by means of the satellite Alouette I, has been discussed by earlier workers^{1, 2}. The cusps are associated with an ionisation ledge lying along the magnetic field line passing through the peaks of the equatorial anomaly.

We here describe observations of cusps associated with echoes reflected from a feature aligned with the field below the anomaly field line and we suggest how the ledge of ionisation in the equatorial topside ionosphere may be formed.

On ISIS-2 ionograms from the dip latitude region 9°N to 7.5°S, recorded at Ahmedabad on October 6, 1972. The ionograms were reduced to electron density profiles by the 'parabolic in log *N* method'³.

Figure 1 shows one of the sequence of ionograms exhibiting a cusp, and Fig. 2 shows the corresponding electron density profile. The ledge, which is an enhancement of the electron density over the 'normal' level shown by the dashed line is clearly evident. The horizontal arrow (Fig. 2) indicates the height of maximum percentage deviation of the electron density in the ledge region and the tip of the vertical arrow indicates the height from which the cusp echoes were reflected. The cusp echoes were returned from below the ledge.

Figure 3 shows the heights of constant electron density contours in an altitude against dip latitude diagram. The equatorial anomaly is well developed and the dashed line in the diagram shows the magnetic field line passing through the peaks of the equatorial anomaly. The height of the anomaly field line over the equator is 860 km. The heights at which the maximum percentage deviation of the ledge electron density

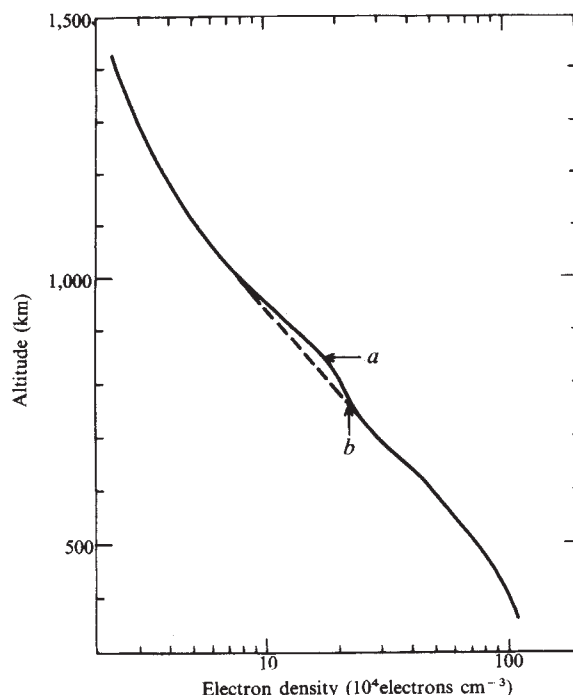


Fig. 2 The electron density profile obtained from the ionogram shown in Fig. 1, recorded October 6, 1972 (UT, 08 h 00 min 32s; LT 1240). Longitude, 70° E; latitude, 7.6° N; dip latitude, -1.42°. *a*, Height of maximum deviation of electron density; *b*, altitude of echoes associated with cusps.

occurred at different dip latitudes are shown by squares in the diagram and they fall on or closely to the anomaly field line. The maximum percentage deviation was observed to increase towards the dip equator, reaching a maximum value of 16%.

The circles in Fig. 3 indicate the heights at which the cusp echoes were reflected. The locus of the 'cusp points' are aligned along a magnetic field line crossing the magnetic equator 775 km above the ground, that is, below the anomaly field line. The ionograms were accurately scaled and the error in evaluating the true height of the cusp points is not more than 15 km. A possible mechanism leading to the formation of a ledge of ionisation such as that observed, can be described.

Recent observations of the neutral densities of molecular

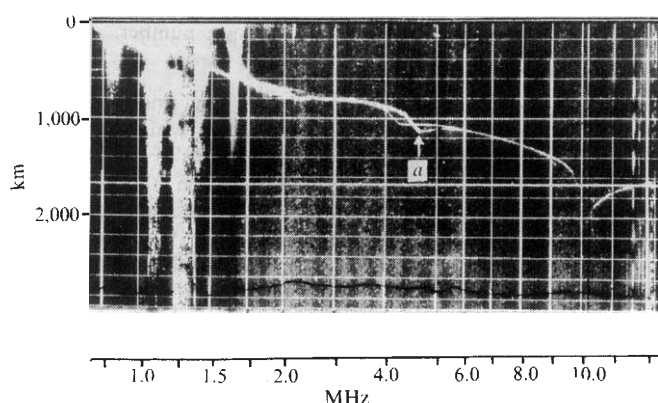


Fig. 1 ISIS-2 topside ionogram showing the cusp (*a*). The *x* axis is the frequency (MHz), the *y* axis is the virtual depth (km) below the satellite.

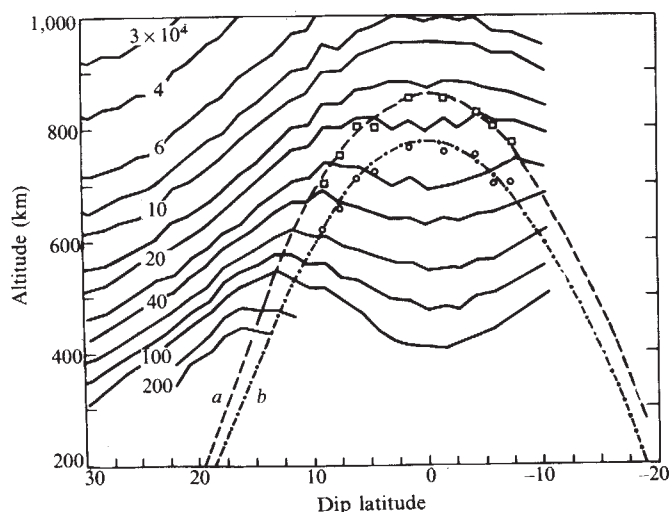


Fig. 3 Latitudinal variation of the height of contours of constant electron density. □, Ledge maxima; ○, heights at which echoes associated with the cusps were reflected. *a*, Locus of anomaly peak; *b*, locus of cusps.

nitrogen and atomic oxygen using a mass spectrometer on the satellite, OGO 6, reveal an equatorial anomaly at an altitude of about 450 km, similar to that in the equatorial ionosphere; density maxima (crests) occur on either side of a minimum (trough) at the magnetic equator. Typical values of the differences between the densities of N_2 and O at the trough from those at the crests, observed at $\pm 20^\circ$ magnetic latitude, are 20% and 10% respectively. It seems likely that the neutral density enhancements will extend above 450 km, along a magnetic field line in a manner similar to the ionisation enhancements, and will merge above the magnetic equator. The neutral temperatures derived from the observed densities show enhancements of 3 to 4% at the neutral density crests.

The existence of enhancements aligned with the field in the neutral density will decrease the diffusion of plasma along the field lines involved. Ionisation in the equatorial ionosphere is lifted vertically during the day by the $\mathbf{E} \times \mathbf{B}$ effect, where $\mathbf{E}$ and $\mathbf{B}$ are the electric and magnetic fields respectively, and diffuses downwards along the magnetic field lines with an ambipolar diffusion coefficient⁴:

$$D_a = [(T_e + T_i)/T_n] [7 \times 10^{18}/n(O)] [T_n/1,000]^{1/2} \text{ cm}^2 \text{ s}^{-1} \quad (1)$$

where T_n , T_i and T_e are the neutral, ion and electron temperatures respectively, and $n(O)$ is the number density of atomic oxygen. The value of D_a increases exponentially with altitude as the neutral density, which is predominantly atomic oxygen in the topside ionosphere, decreases, and can be represented by $D_a = D_0 e^z$ where z is measured in units of the atmospheric scale height H and D_0 is the value of the diffusion coefficient at any known lower level.

The ionisation velocity, V , along the field line, resulting from diffusion in the absence of temperature gradients and winds, is given by⁵

$$-V = (D_a/H) [(1/N)(dN/ds) - (1/2) \sin I] \quad (2)$$

where I is the dip angle and s the distance along the field line measured in units of H and taken positive in the northward direction, so that $-dz/ds = \sin I$.

Equation 2 shows that at a given dip angle V is directly proportional to D_a which varies inversely with the density of neutral atomic oxygen (equation 1). Away from the equator neutral density enhancements occur along the ionisation anomaly field line. Along this field line the diffusion coefficient, and therefore the diffusion velocity parallel to the field line, will be decreased by about 10% because of the enhanced O concentration. Any increase in neutral temperature along the same field line will increase the scale height. This further decreases the diffusion velocity. We suggest that these processes will result in the enhancement of the electron density around the field line on which the neutral density is greatest. The field line with which the ionogram cusps are associated may thus occur at the beginning of the sharp rise in the neutral density.

We thank Professor K. R. Ramanathan for helpful discussions. This work was supported by the Department of Space, Government of India.

R. RAGHAVARAO
M. R. SIVARAMAN

Physical Research Laboratory,
Ahmedabad, India.

Received December 10, 1973

¹ Lockwood, G. E. K., and Nelms, G. L., *J. atmos. terr. Phys.*, **26**, 569 (1964).

² King, J. W., Smith, P. A., Eccles, D., Fooks, G. F., and Helm, H., *Proc. R. Soc.*, **A281**, 464 (1964).

³ Jackson, J. E., *Proc. IEEE*, **57**, 960 (1969).

⁴ Hedin, A. E., and Mayr, H. G., *J. geophys. Res.*, **78**, 1688 (1973).

⁵ Rishbeth, H., and Garriott, O. K., *Introduction to Ionospheric Physics*, 144, (Academic Press, New York and London 1969).

Lightning incidence in Britain and the solar cycle

The existence of a correlation between solar activity and meteorological or climatological parameters seems no longer in doubt and a credible explanation has recently been suggested¹. It has also been shown that the mean monthly or yearly number of sunspots gives a crude but effective index of the solar activity and that the most marked variation is cyclic with a period of approximately 11 yr.

It has been deduced from lightning-counter records (F. Popolansky's, unpublished) and from analysis of faults caused by lightning in overhead electricity supply systems^{2,3} that the mean incidence of lightning (usually expressed as the number of flashes per unit area per year) is proportional to the mean number of annual thunderstorm days recorded by observers, raised to the power 1.9. The data from 40 Meteorological Office observers chosen to be representative of Britain were used to obtain means of the annual number of thunderstorm days for the years 1930 to 1973, and the square of the mean for each year was taken as a rough index of the annual incidence of lightning. The 5-yr running means of both the annual values of this index and the mean annual sunspot numbers are plotted in Fig. 1. In spite of the year-to-year variations in

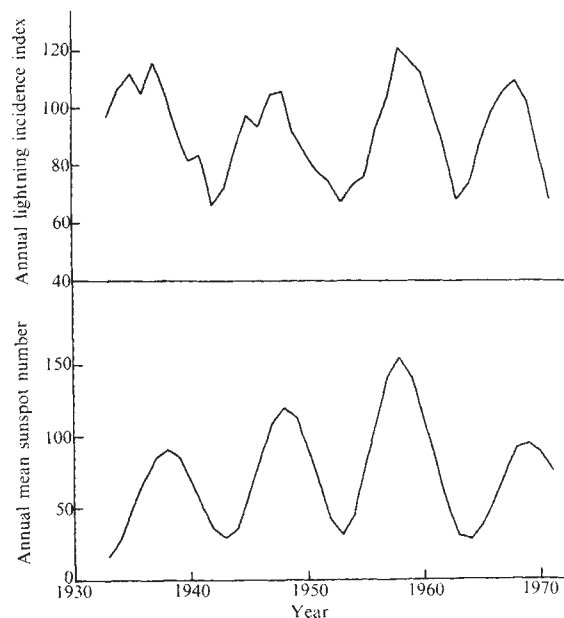


Fig. 1 Annual variation of: a, 5-yr running means of lightning incidence; b, sunspot number.

the incidence of lightning, there is an underlying cyclic variation with a period of about 11 yr which is in phase with the solar cycle and has an amplitude of $\pm 30\%$ of the mean. The correlation obtained between these smoothed data is 0.8, a result of statistical significance despite the smoothing. During the period 1964 to 1972 the trend in faults attributed to lightning on the British electricity distribution network also followed the lightning incidence/sunspot cycles but these fault data are too few to be statistically significant.

The conclusion that the underlying trend in lightning activity is related to the solar cycle is not only of interest to the atmospheric physicist but also to those electricity supply and civil engineers who are concerned with long-term planning of lightning protection. Of more immediate

interest is the fact that mean lightning incidence in Britain reached a minimum in 1973 and, neglecting year-to-year variations, can be expected to reach a peak at the peak of the next solar cycle with a magnitude of about twice the 1973 level.

M. F. STRINGFELLOW

Electricity Council Research Centre
Capenhurst, Chester CH1 6ES, UK

Received March 6, 1974.

¹ King, J. W., *Nature*, **245**, 443–446 (1973).

² Forrest, J. S., *Q. J. L. R. meteor. Soc.*, **329**, 277–286 (1950).

³ Stringfellow, M. F., *Electricity Council Report*, No. ECRC/R602 (1973).

Major and trace elements in the Allende meteorite

WE have determined major and trace element abundances for seven Ca,Al-rich aggregates, ten melilite chondrules, and an olivine chondrule and two olivine-rich aggregates from the Allende meteorite, a type III carbonaceous chondrite. Major element abundances were determined with the electron microprobe technique described by Reed and Ware. An MS7 spark source mass spectrometer was used for the determination of trace element abundances. The technique used was that described by Taylor² but with improved and extended data reduction techniques. The precision is about $\pm 5\%$ for the rare earth elements (REEs) and the accuracy about $\pm 10\%$, but varies somewhat, being poorer for elements below mass number 105 than for those with higher mass numbers.

The Allende samples can be grouped in various ways using chemical, mineralogical and textural parameters. Our

results distinguish four distinct groups, as follows: group I, melilite-rich chondrules; group II, Ca,Al-rich aggregates with fractionated chondrite-normalised REE abundance patterns; groups III, Ca,Al-rich aggregates with unfractionated chondrite-normalised REE abundance patterns, except for negative Eu and Yb anomalies; group IV, olivine-rich chondrules and aggregates.

The range and average of the elements studied for each group and for the bulk meteorite are given in Table 1. Chondrite-normalised REE abundances of the average for each group and for the bulk meteorite are shown in Fig. 1.

The melilite-rich chondrules in group I are spherical aggregates of melilite, Ti-rich fassaite, spinel and anorthite with a coarsely crystalline igneous texture, and have high Al_2O_3 , high CaO and an unfractionated REE pattern averaging 10–15 times chondritic abundances but with a small positive Eu anomaly. In contrast the olivine-rich chondrules and aggregates of group IV have an REE abundance pattern averaging three times that of the chondrites, with possibly a slight positive Ce anomaly and a slight negative Eu anomaly. The major and trace element abundances of these two chemically distinct types of chondrules indicate that the melilite chondrules may represent the initial condensates from a cooling gas of solar composition and the olivine chondrules later low-temperature condensates. (Condensation sequences for a cooling gas of solar composition have been calculated by Lord³, Larimer⁴ and Grossman⁵.)

The fine-grained Ca,Al-rich aggregates, composed mainly of spinel, fassaite, melilite and/or grossular, and minor amounts of nepheline and sodalite, can be divided into two groups on the basis of their trace element abundances. Two of the seven examined, classified as group III, have relatively unfractionated REE abundances at about 20 times those of chondrites, but with large negative Eu and Yb anomalies. Even though the chemistry of these aggregates, like that of the melilite chondrules, suggests they would be the first condensate from a cooling gas of solar composition,

Table 1 Chemical composition (oxides in weight per cent, elements in p.p.m.) for selected materials from the Allende meteorite.

	Group I (10)		Group II (5)		Group III (2)		Group IV (3)		Bulk
	Range	Average	Range	Average	Range	Average	Range	Average	
SiO ₂	24.9–35.1	30.0	17.7–34.5	28.9	20.5,24.5	22.5	38.8–45.2	42.0	34.28
TiO ₂	1.0–1.5	1.2	0.30–1.1	0.64	0.74,0.96	0.85	0.17–0.30	0.22	0.15
Al ₂ O ₃	21.5–32.6	28.6	19.3–44.0	30.3	43.3,40.3	41.8	3.4–9.4	5.7	3.29
FeO	0.4–3.1	1.7	1.3–12.2	7.1	3.7,3.9	3.8	9.6–11.0	10.5	27.15
MgO	6.9–12.6	9.9	10.1–16.6	12.8	15.4,13.6	14.5	23.5–36.6	31.8	24.63
CaO	23.3–33.3	27.7	8.6–24.0	13.2	11.4,11.4	11.4	2.7–8.3	4.7	2.59
Na ₂ O	0.11–0.92	0.38	0.39–4.7	3.1	1.3,1.8	1.6	0.45–2.7	1.5	0.46
K ₂ O	0.00–0.08	0.02	0.02–0.26	0.12	0.04,0.05	0.05	0.03–0.20	0.09	0.03
Rb	0.05–0.97	0.33	0.42–6.1	3.2	1.2,1.1	1.2	1.1–20	6.9	1.3
Sr	110–200	136	18–73	37	28,43	35	13–20	18	12
Y	15–29	23	0.86–3.0	1.7	36,41	39	1.6–4.3	3.5	3.1
Zr	35–72	56	1.9–7.2	4.1	99,113	106	3.8–11	8.4	10
Nb	1.9–5.1	3.7	0.94–2.6	1.7	1.9,9.1	5.5	0.54–0.89	0.77	0.65
Mo	7.4–14	11	<0.7	<0.7	4.2,9.8	7.0	0.9–1.1	1.0	1.5
Ba	23–104	57	6.2–22	15	22,10	16	5.3–9.8	9.0	4.1
La	3.2–4.7	4.1	3.7–13	7.8	6.5,5.8	6.2	0.79–1.9	1.2	0.51
Ce	8.2–12	11	10–31	19	11,15	13	2.5–4.8	4.0	1.3
Pr	1.2–1.9	1.6	1.6–4.7	3.0	2.2,2.1	2.2	0.32–0.72	0.45	0.21
Nd	5.4–9.0	6.8	6.2–20	13	11,9.9	10	1.6–3.1	2.1	0.97
Sm	1.7–3.2	2.2	2.2–6.2	4.0	3.1,3.1	3.1	0.49–1.1	0.73	0.34
Eu	0.76–1.3	1.0	0.17–0.62	0.28	0.25,0.36	0.31	0.13–0.19	0.16	0.10
Gd	2.2–4.0	3.1	0.84–3.8	2.3	4.5,4.6	4.6	0.46–1.1	0.75	0.42
Tb	0.41–0.71	0.53	0.12–0.52	0.31	0.81,0.82	0.82	0.08–0.17	0.12	0.08
Dy	2.5–4.8	3.7	0.66–2.6	1.6	6.5,6.5	6.5	0.53–0.92	0.75	0.42
Ho	0.59–1.2	0.94	0.07–0.21	0.14	2.3,1.7	2.0	0.13–0.19	0.16	0.10
Er	1.9–3.8	2.8	0.15–0.43	0.26	6.0,4.9	5.5	0.38–0.60	0.47	0.29
Tm	0.30–0.46	0.38	<0.2	<0.2	0.74,0.71	0.73	<0.2	<0.2	0.05
Yb	2.1–3.4	2.6	0.37–1.6	0.81	0.74,1.9	1.3	0.47–0.70	0.60	0.31
Hf	0.83–1.5	1.3	<0.3	<0.3	2.2,2.5	2.4	<0.3	<0.3	0.2
Th	0.34–0.61	0.45	0.14–0.72	0.46	0.70,0.72	0.71	0.06–0.17	0.12	0.07

The numbers in brackets after the groups are the number of specimens analysed; the figures for the bulk composition are from a compilation by E. Jarosewich (unpublished).

it is apparent from their fine-grained texture and the presence of grossular that these aggregates cooled rapidly, certainly more rapidly than the primeval solar nebula. It would seem therefore that the melilite chondrules and the aggregates of Group III have been subjected to similar high energy events, which vaporised already condensed material, the chondrules cooling from a gas through the liquid state to the solid whereas the aggregates, because of conditions of lower pressure, cooled directly from a gas to a fine-grained solid.

The remaining five Ca,Al-rich aggregates, however, classified as Group II, exhibit remarkable REE abundance patterns. Although the light REE (La-Sm) show similar abundances in groups II and III, group II aggregates have rapidly diminishing abundances of the heavier REEs. Superimposed upon this are a marked negative Eu anomaly and a large positive Yb anomaly. Group II aggregates are also depleted in Y, Zr, Mo and possibly Nb with respect to those of group III. Tanaka and Masuda⁶ have described an Allende aggregate with a group II REE pattern. Like the group III Ca,Al-rich aggregates, the aggregates of group II have a fine-grained texture and contain metastable minerals, indicating they cooled rapidly, probably directly from the gas phase to a fine-grained solid.

It is very difficult to produce the fractionated REE abundance patterns of group II either by fractional condensation or evaporation at a suitable temperature range based solely on the boiling points of the REE metals or any of their compounds for which there are sufficient data. For example, even assuming that Eu and Yb exist in the divalent state and are located in different lattice sites to the remaining trivalent REEs, Sm and Dy are not depleted relative to the other REEs yet have much lower boiling points. It is possible that a property of the REEs for which we, as yet, do not have sufficient information might, under certain conditions, give rise to the fractionated REE patterns observed.

An intriguing alternative is that the group II aggregates may contain a component which has had a different history of nucleosynthesis to other matter in the Solar System. The light REEs are produced by an *s* process inside stars whereas the heavy REEs are produced predominantly by the *r* process during supernova explosions. Clayton *et al.*⁷ on the basis of oxygen isotope anomalies, have postulated a separate history of nucleosynthesis for some of the Ca, Al-rich material of the Allende meteorite. They found

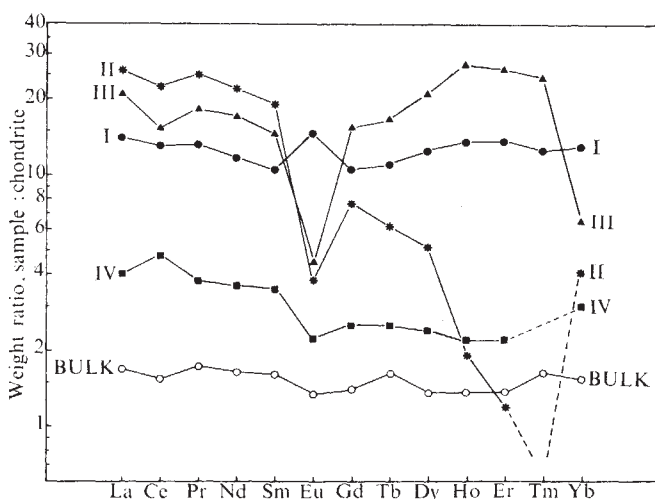


Fig. 1 Chondrite-normalised REE abundance patterns of the average for each group and of a bulk sample of the Allende meteorite. Group I: melilite-rich chondrules; groups II and III: Ca,Al-rich aggregates; group IV: olivine-rich chondrules and aggregates. Note the Eu and Yb abnormalities in groups II and III.

enrichment of ¹⁶O in all anhydrous minerals in C2 and C3 meteorites, however, whereas we find depletion of heavy REEs only in group II Allende aggregates, and thus there seems to be no correlation between these two anomalies. Critical examination of our data on isotopic abundances of the REE shows no indication of preferential depletion in *r*-process nuclides, and Gray *et al.*⁸ found no evidence of lack of isotopic homogeneity in Rb and Sr except for variation in ⁸⁷Sr abundance produced by radioactive decay of ⁸⁷Rb.

The differences in minor and trace element distributions evidently reflect differing conditions of genesis of these components. The melilite chondrules seem to represent material segregated at an early high temperature stage; the olivine chondrules and aggregates at a slightly later stage, and the aggregates of groups II and III may represent residual material which condensed after the previous components had segregated from the initial material. The complex pattern of trace element distributions in these Allende components indicates a complex history of formation of this meteorite.

We thank Mrs P. Muir, Mr J. Nelen, Mr M. P. Gorton and Professor A. E. Ringwood for help and advice. This research was supported by the National Aeronautics and Space Administration and the Smithsonian Research Foundation.

PHILIP M. MARTIN

Research School of Earth Sciences,
Australian National University,
Canberra, ACT, Australia

BRIAN MASON

Mineral Sciences,
Smithsonian Institution,
Washington, DC 20560

Received January 17; revised March 4, 1974.

- ¹ Reed, S. J. B., and Ware, N. G., *X-Ray Spectrom.*, **2**, 69 (1972).
- ² Taylor, S. R., *Geochim. cosmochim. Acta*, **35**, 1187 (1971).
- ³ Lord, H. C., *Icarus*, **4**, 279 (1965).
- ⁴ Larimer, J. W., *Geochim. cosmochim. Acta*, **31**, 1215 (1967).
- ⁵ Grossman, L., *Geochim. cosmochim. Acta*, **36**, 579 (1972).
- ⁶ Tanaka, T., and Masuda, A., *Icarus*, **19**, 523 (1973).
- ⁷ Clayton, R. N., Grossman L., and Mayeda, T. K., *Science*, **182**, 485 (1973).
- ⁸ Gray, C. M., Papanastassiou, D. A., and Wasserburg, G. J., *Icarus*, **20**, 213 (1973).

Chandler wobble and viscosity in the Earth's core

JEFFREYS¹, and Munk and MacDonald², have stated that viscosity of the core cannot account for the observed damping of the Chandler wobble. This statement may be in error.

Jeffreys's statement is based on a simple algebraic error on page 257 of the fourth edition of *The Earth*¹. He starts from the assumption that there exists, at the core-mantle boundary, an Ekman boundary layer of thickness $(\nu/\omega)^{1/2}$, where ν is the kinematic viscosity and ω the angular velocity of rotation of the earth. If V is the differential velocity between the core and the mantle, the rate of dissipation for unit area is of the order of $\rho V^2 (\nu/\omega)^{1/2}$, where ρ is the density. If α is the small angle between the axes of rotation of core and mantle, $V = \alpha a \omega$, where a is the radius of the core. The total rate of dissipation, F , is then

$$F = 4\pi \rho a^4 \omega^2 \alpha^2 (\nu/\omega)^{1/2} \quad (1)$$

This is Jeffreys's equation (13) except for the factor 4π and his inadvertent writing of ω^3 instead of ω^2 . This error is unfortunately carried into the numerical result on the following line (Jeffreys's equation (14)); the damping time, which is proportional to $1/F$, is consequently too long.

Munk and MacDonald² (page 170) proceed in essentially the same way as Jeffreys, except that they write $V = \alpha\omega$, substituting the frequency, ω , of the Chandler wobble for ω . The substitution is hard to justify. Munk and MacDonald justify it with Jeffreys and Vicente's³ demonstration that, to a first approximation, the core remains motionless, which is, however, precisely the reason that Jeffreys¹ adopted $V = \alpha\omega$.

Returning to Jeffreys' corrected expression (1), and comparing F with the wobble energy, E_w of the mantle:

$$E_w = \frac{1}{2} A_m \frac{(C_m - A_m)}{C_m} \omega^2 \alpha^2 \quad (2)$$

where A_m and C_m are the principal moments of inertia of the mantle. The time, τ , needed to reduce the energy by $1/e$ is

$$\tau = \frac{E_w}{F} = \frac{A_m}{8\pi\rho a^4} \frac{(C_m - A_m)}{C_m} (v\omega)^{-1} \quad (3)$$

and the time needed to reduce the amplitude in the same ratio is 2τ . Observations indicate that $2\tau \sim 30$ yr. Equation 3 then gives for the kinematic viscosity of the core

$$\nu = 0.026 \text{ cm}^2 \text{ s}^{-1} \quad (4)$$

Leppaluoto⁴, using the significant-structure theory of liquids, finds that the dynamic viscosity, $\eta = \nu\rho$, of liquid iron at its melting point and at the pressure of the core-mantle interface (1.4 Mbar) probably lies between 0.1, and 0.5 poise. The density at the top of the core is approximately 10 g cm^{-3} , and the corresponding limits on ν are therefore 0.01 and $0.05 \text{ cm}^2 \text{ s}^{-1}$, which nicely bracket the value of equation 4, even though Leppaluoto's values should be corrected for the presence in the core of elements other than iron (for example, sulphur), and for temperature, which, at the core-mantle boundary, is unlikely to be precisely the melting temperature of pure iron. Gans⁵, using somewhat questionable approximations, suggests $\nu = 0.066 \text{ cm}^2 \text{ s}^{-1}$ as a typical value of the viscosity of the liquid core at the inner-core boundary.

The third (1952) edition of *The Earth* does not have the ω^3 error⁶. Assuming $\nu = 5 \times 10^7 \text{ cm}^2 \text{ s}^{-1}$, Jeffreys consequently found a damping time that was too short and concluded that the viscosity of the core must be less than $10^5 \text{ cm}^2 \text{ s}^{-1}$ to account for the observed damping. He did not conclude at that time that viscous damping of the Chandler wobble is impossible, as indeed it does not seem to be.

J. VERHOOGEN

Department of Geology,
University of California,
Berkeley 94720

Received January 18, 1974.

¹ Jeffreys, H., *The Earth*, fourth ed. (Cambridge University Press, Cambridge, 1959).

² Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth* (Cambridge University Press, Cambridge, 1960).

³ Jeffreys, H., and Vicente, R., *Mon. Not. R. astr. Soc.*, **117**, 142 (1957).

⁴ Leppaluoto, D., thesis, Univ. California, Berkeley (1972).

⁵ Gans, R. F., *J. geophys. Res.*, **77**, 360 (1972).

⁶ Jeffreys, H., *The Earth*, third ed. (Cambridge University Press, Cambridge, 1952).

Montmorillonite in surface detritus

QUANTITATIVELY, clay minerals are usually the most important components of those fractions of deep-sea sediments which are smaller than $2 \mu\text{m}$. There are specific patterns in the distributions of the principal groups of clay minerals in both deep-sea sediments¹ and in aeolian dusts from the overlying marine atmosphere², which have been used to evaluate the origin of the clays. It is considered¹⁻³ that the clay minerals kaolinite, illite and chlorite are largely detrital in origin and are transported to the oceans from the continents.

Less is known, however, about the origin of montmorillonite. Deep-sea sediments of some regions, for example, the South Pacific, show extensive evidence that montmoril-

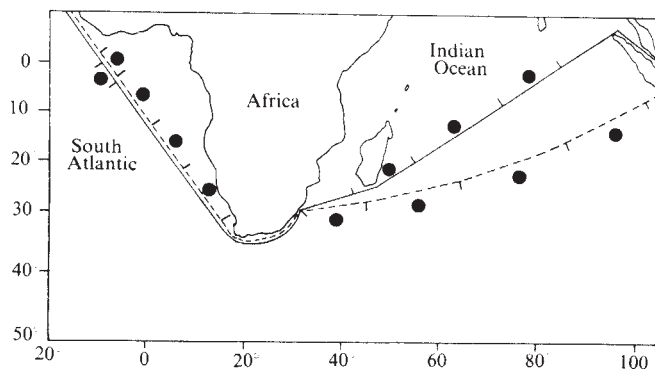


Fig. 1 Partial track of the MV Cyclops. Solid line, UK-Japan run; broken line, Japan-UK run. Detritus samples were collected over the intervals indicated by the lines perpendicular to the track; ●, central positions of sampling sites.

lonite has formed from the *in situ* alteration of volcanic debris on the ocean floor¹, that is the montmorillonite is authigenic. A similar explanation has been proposed for the origin of montmorillonite in Indian Ocean deep-sea sediments¹, where this mineral makes up approximately 41% of the total clays smaller than $2 \mu\text{m}$. There is, however, speculation about the origin of montmorillonite in deep-sea sediments from the South Atlantic, where it comprises approximately 26% of the total clays smaller than $2 \mu\text{m}$ (ref. 1). It is believed¹⁻³ that either the montmorillonite or the parent volcanic material is transported to the region by aeolian and river mechanisms or that the mineral is derived from volcanic material from the Mid-Atlantic Ridge region. These theories are based on observations of the distribution of montmorillonite in deep-sea sediments; there is little information available on its concentration in marine detritus from the World Ocean.

Here, we present preliminary data on the relative concentrations of montmorillonite in 'open-ocean' surface water samples (0-5 m) from the South Atlantic and Indian Oceans. Collections were made during March and July, 1972 on board MV Cyclops by pumping water from a position in the ship's bows (which minimised contamination from the ship's sides) through plastic tubing. The particulate matter was separated by continuous centrifuging. The centrifuge bowl was lined with plastic and after each collection the liners were removed, placed in plastic bags and stored in a deep freeze. The detritus was later removed from the liners and dried at about 40°C , and representative samples taken. A fraction of the samples smaller than $2 \mu\text{m}$ was separated, treated with hydrogen peroxide to remove organic matter, and the clay minerals were determined by X-ray diffraction. The ship's track and the sampling positions are shown in Fig. 1 and the average montmorillonite concentrations in the detritus are listed in Table 1 together with those of the underlying deep-sea sediments.

On average, the concentration of montmorillonite is similar in detritus samples from both the South Atlantic and Indian Oceans, and constitutes approximately 14% of the total clays smaller than $2 \mu\text{m}$. This is considerably less montmorillonite than is present in the underlying deep-sea sediments. Furthermore, the relative distributions of montmorillonite vary between the detritus and deep-sea sediments of the two regions; in the South Atlantic there is about one and a half times as much montmorillonite in the sediments as in the detritus, and in the Indian Ocean about three times as much in the sediments.

There are a number of explanations for the deficiency of montmorillonite in the detritus relative to the sediments. For example, other clay mineral species, and/or volcanic

Table 1 Average concentration of montmorillonite*

South Atlantic Ocean	
Detritus	~ 14%
Deep sea sediments ²	~ 26%
Indian Ocean	
Detritus	~ 14%
Deep sea sediments ¹	~ 41%

* The concentrations of montmorillonite are given as a percentage of the four clay minerals montmorillonite, illite, chlorite and kaolinite in the fraction of the detritus and sediments smaller than 2 μ m.

ash, could be transported to the surface of the ocean and could subsequently undergo modification to form montmorillonite both in the water column and at the sediment surface. Alternatively, montmorillonite could be formed from the *in situ* modification of locally derived volcanic debris at the sediment surface. Whatever processes are operative, however, it may be concluded from the present results that only about half of the montmorillonite in South Atlantic deep-sea sediments, and only about a third of that in Indian Ocean sediments, have a continental detrital origin.

We thank the Blue Funnel Line for permission to make collections from their ships; the Master, officers and crew of the MV Cyclops; A. J. Woodward for his help in the collection of samples; and the Natural Environment Research Council for financial support.

R. CHESTER
J. H. STONER

Department of Oceanography,
The University,
Liverpool, L69 3BX, UK

L. R. JOHNSON

c/o British Museum (Natural History),
Cromwell Road,
London SW7 5BD, UK

Received February 20, 1974.

¹ Griffin, J. J., Windom, H., and Goldberg, E. D., *Deep-Sea Res.*, **15**, 433 (1968).

² Chester, R., Elderfield, H., Griffin, J. J., Johnson, L. R., and Padgham, R. C., *Mar. Geol.*, **13**, 91 (1972).

³ Biscaye, P. E., *Yale Univ., Geochem. tech. Rep.*, **8** (1964).

Revised continental fit of Australia and Antarctica

I HAVE already presented a morphological reconstruction of the continental fragments of Gondwanaland in the south-western Pacific¹. The reassembly was based on the computer-generated fits of Australia and Antarctica^{2,3} (which match the 1,000-fathom and 500-fathom isobaths). The method used in each case^{2,3} is a 'least squares' geometric fit of the selected isobath. Another fit has recently been proposed⁴, for this fit an instantaneous rotation pole is determined from fracture zones, at 10.8° N, 32.2° E, but the Australia–Antarctica fit is based on a 'least squares' geometric pole at 6° S, 40.5° E. I suggest that all of these fits may be in error by about 250 km, as present-day isobaths only define poorly the pre-drift continental edges.

A better method of making continental reassemblies is to 'reverse' seafloor spreading, using a mapped pattern of magnetic lineations and fracture zones. Such a pattern is fairly well defined between Australia and Antarctica^{5,6}. Weissel and Hayes⁶ show several stages of drift (see their Fig. 13), but in the initial step they have accepted the computer fits^{2,3} as the constraint. They therefore delineate

a westward bend in fracture zones south of Australia, amounting to some 250 km (see their Fig. 2). Hayes and Ringis⁷ attempted a regional continental reconstruction but erroneously implied that the Australia–Antarctica fit has been "rather precisely determined" using only the reversal of the magnetic patterns, whereas Weissel and Hayes^{5,6}, in fact, used the computer fit^{2,3}. They note that the South Tasman Rise overlaps the Iselin Plateau, presuming that both are continual crust, and then suggest that relative movement of West and East Antarctica occurred during drift.

In the light of these problems and inconsistencies, I have revised my original reconstruction (Fig. 1). The fit was made using a 1:15 million scale Lambert's Equal Area Azimuthal projection, centred at 160° E, 60° S (near Macquarie Island). Continental boundaries were initially defined at the 2,000-m isobath, and fracture zones plotted from ref. 6, except that each fracture zone was projected straight towards the Australian coast. Australia was then

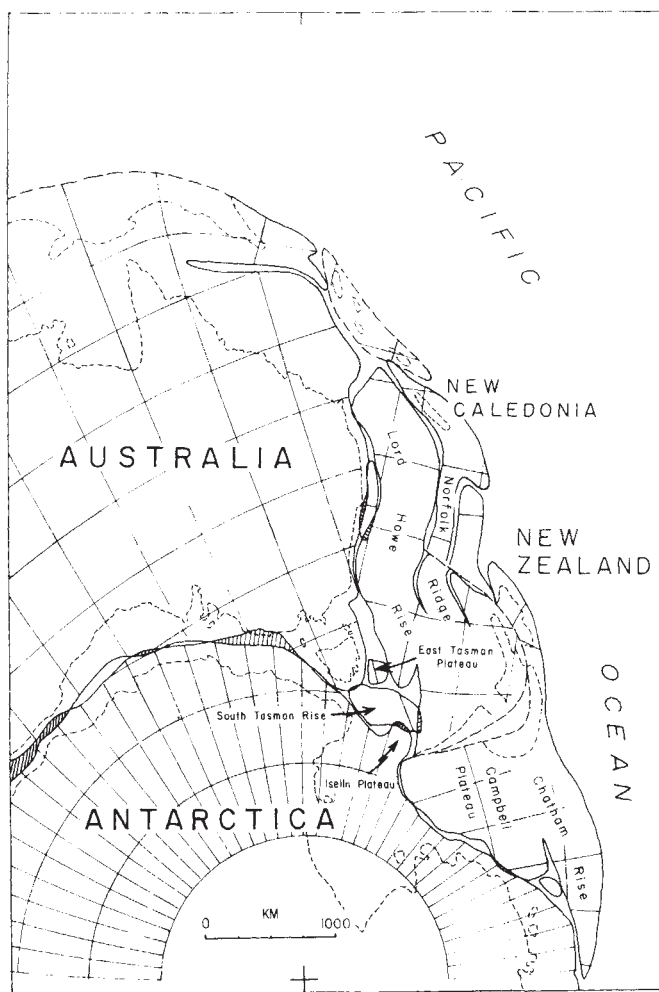


Fig. 1 Reconstruction of continental fragments in the south-western Pacific. Continental outlines and 2,000-m isobaths (smoothed) shown, taken from Lambert's Equal Area Azimuthal projection, centred at 160° E, 60° S. Present latitude and longitude lines (5° spacing) shown for reference. Hatching denotes overlap.

fitted to Antarctica by reversal of motion along the intervening fracture zones. This operation places Australia some 250 km west (relatively) of its position in earlier computer fits²⁻⁴ and allows the continental South Tasman Rise⁸ to fit neatly between Tasmania and the Iselin Plateau (Fig. 1).

The fit was checked by rotating contiguous points to their present positions using a 50-cm stereographic net, and it was found that rotation about a pole at 10.8° N, 32.2° E

(determined from fracture zones in ref. 4) gave results consistent with the fit proposed here, and significantly different from the least squares geometric fits. The shape of mutually opposed coasts on the Lambert's Equal Area projection were compared with their computer-drawn equivalents by enlarging the latter optically to a scale of 1:15 million. No significant distortion was found.

The Campbell Plateau was fitted against Antarctica as before¹ and the Tasman Sea closed by a 23° rotation of the Lord Howe Rise about a rotation centre at 145° E, 15° S. This is broadly consistent with the Tasman Sea magnetic pattern but implies that the orientation of fracture zones, particularly in the north, is different from that of Hayes and Ringis⁷. This suggests that their interpretation may be in error in this region. A 5° grid (present coordinates) is superimposed on the fragments in the reconstruction (Fig. 1). This shows that neither the Campbell Plateau nor the Lord Howe Rise have been deformed to achieve the fit. The only significant deformation required is along the Alpine Fault Zone through New Zealand.

The modified fit (Figs 1 and 2) still has one major overlap, where the bulge of the Antarctic 2,000-m isobath overlaps Australia. A similar mismatch (though placed further west) was noted by Sproll and Dietz², who suggested that either the Antarctic bathymetry was substantially in error in this area, or that postdrift volcanics or sediments form the bulge. A detailed compilation of available oil-company exploration data from the south-eastern Australian continental margin shows, however, that with the fit suggested here, this bulge largely overlaps an Upper Mesozoic and Cainozoic sedimentary sequence in the Otway

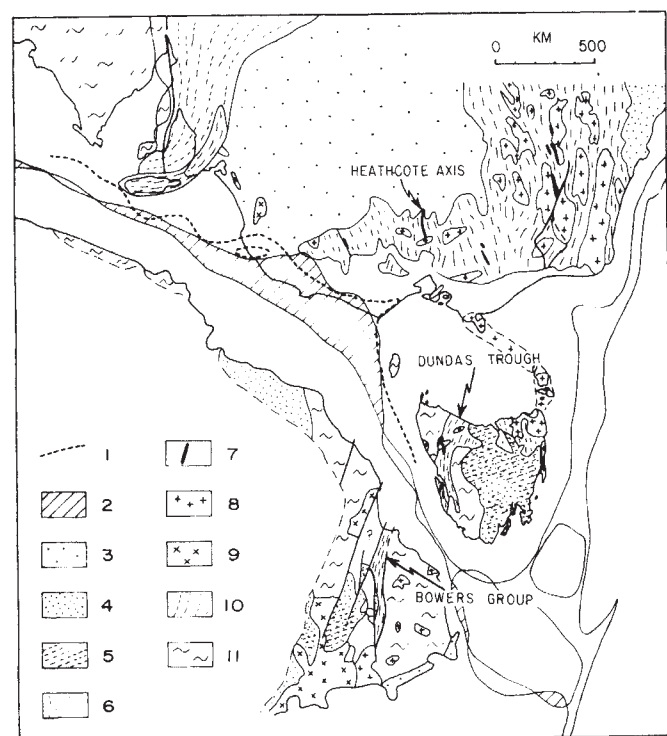


Fig. 2 Detailed fit of south-eastern Australia and Antarctica, showing major geological elements. 1, 3,000-m isobath of Upper Mesozoic Cainozoic sediments of south-eastern Australia; 2, overlap of 2,000-m isobaths; 3, Cainozoic sedimentary cover; 4, Upper Palaeozoic and Mesozoic basins (essentially undeformed sediments); 5, areas of Jurassic dolerite intrusions; 6, Palaeozoic of Tasman Orogenic Zone; 7, ultramafic masses; 8, Silurian-Devonian granites; 9, Cambrian-Ordovician granites; 10, late Proterozoic sediments of the Adelaide Geosyncline (moderately folded); 11, exposed Precambrian "basement". Some major faults are also shown by heavier solid lines. Data taken from refs 11 (Australia) and 12 (Antarctica).

Basin of south-eastern Australia⁹. These are typical rift-fill sediments (refs 9, 10, and K. A. Richards, and B. M. Hopkins, ECAFE Symposium, Canberra, 1969) which represent an unusually wide development of the continental margin in this area. On Fig. 2, I have delineated the 3,000-m isobath of Upper Mesozoic and Cainozoic sediments along the Australian margin, selecting this line as more representative of the predrift margin. The correspondence of this isobath with the Antarctic 'bulge' is quite clear.

The outlined fit (Figs 1 and 2) is therefore preferred to computer fits based on present isobaths^{2,3} and points to the obvious need to establish the geological status of lines of fit before using computer techniques. Isobaths of marginal sediments are probably a more realistic definition of predrift continental shapes. The fit also eliminates the source of several 'problems' in the region. For example, the South Tasman Rise is incorporated without any movement of West Antarctica.

Figure 2 also shows the major geological elements of mainland Australia, Tasmania and Antarctica^{11,12}. No certain correlations can be made yet. One speculative correlation, however, is that of the Dundas Trough (Cambrian sediments, ophiolites and Andean-type calc-alkaline volcanics¹³) with the Heathcote Axis (Cambrian greenstones and calc-alkaline volcanics¹⁴) and Bowers Group (Cambro-Ordovician sediments^{15,16}). This suggestion offers a chance to correlate narrow units, without depending on vague lithological similarities, but needs further evaluation.

There has been some discussion of alternative reassemblies and argument for and against major transcurrent movements active during the Palaeozoic and Mesozoic¹⁷⁻²⁰. These articles do not allow for any possible error in the computer fits^{2,3} and generally make very vague and generalised lithological correlations to support their conclusions. Many of the supposed difficulties in regional correlations using earlier fits^{2,3} diminish considerably if the revised fit presented here is valid. The occurrence of Precambrian basement rocks in Tasmania also suggests that the island is uplifted relative to Victoria and Antarctica, probably as a result of Cainozoic seafloor spreading and therefore a relatively deeper erosion level is observed at present in Tasmania⁹.

The present knowledge of the regional geology, seems to provide little to support unequivocally hypotheses involving major transcurrent movements^{17,19,20}.

I thank R. D. Beattie for helpful comments.

J. R. GRIFFITHS

Geology Department,
University of Tasmania,
Hobart, Australia 7001

Received October 15, 1973; revised March 19, 1974.

- ¹ Griffiths, J. R., *Nature*, **234**, 203 (1971).
- ² Sproll, W. P., and Dietz, R. S., *Nature*, **222**, 345 (1969).
- ³ Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
- ⁴ McKenzie, D., and Sclater, J. G., *Geophys. J. R. astr. Soc.*, **24**, 437 (1971).
- ⁵ Weissel, J. K., and Hayes, D. E., *Nature*, **231**, 518 (1971).
- ⁶ Weissel, J. K., and Hayes, D. E., *Antarctic Oceanology II: the Australian-New Zealand Sector*, *Antarctic Research Series*, **19**, 165 (American Geophysical Union, Washington, 1972).
- ⁷ Hayes, D. E., and Ringis, J., *Nature*, **243**, 454 (1973).
- ⁸ Kennett, J. P., et al., *Geotimes*, **18**, 14 (1973).
- ⁹ Griffiths, J. R., thesis, Univ. Tasmania (1973).
- ¹⁰ Griffiths, J. R., *J. Austr. Petrol. Explor. Ass.*, **11**, 75 (1971).
- ¹¹ *Tectonic Map of Australia and New Guinea 1:5 000 000* (Geological Society of Australia, 1971).
- ¹² *Geologic Map of Antarctica 1:5 000 000* (American Geographical Society, 1972).
- ¹³ Solomon, M., and Griffiths, J. R., *Proceedings of the Tasman Geosyncline Symposium, Brisbane* (in the press).
- ¹⁴ Talent, J. E., and Thomas, D. E., *Regional Guide to Victorian Geology, Second Edition* (edit. by McAndrew, J., and Marsden, M. A. H.), 65 (University of Melbourne, Melbourne, 1973).

- ¹⁵ Sturm, A., and Carryer, S. J., *N.Z. J. Geol. Geophys.*, **13**, 408 (1970).
¹⁶ Laird, M. G., Andrews, P. B., Kyle, P., and Jennings, P., *Nature*, **238** 34 (1972).
¹⁷ Crawford A. R., and Campbell, K. S. W., *Nature*, **241**, 11 (1973).
¹⁸ Dally, B., Jago, J. B., and Milnes, A. R., *Nature*, **244**, 61 (1973).
¹⁹ Crawford, A. R., and Campbell, K. S. W., *Nature*, **244**, 64 (1973).
²⁰ Harrington, H. J., Burns, K. L., and Thompson, B. R., *Nature*, **245**, 109 (1973).

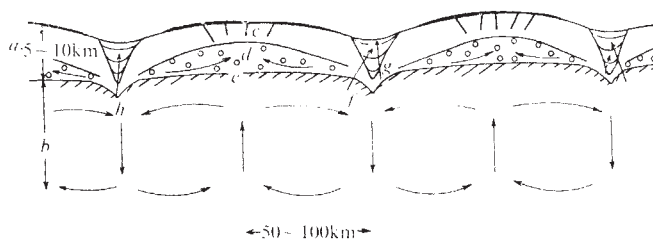


Fig. 1 Model of convective motion in the early crust and upper mantle, a, granitic crust; b, low velocity zone of upper mantle; c, region of basaltic penetration of the crust; d, granitic domes; e, granulites and anorthosites; f, basalts; g, rhyolites; h, andesites.

Archaean tectonics

THERE is compelling evidence that Archaean convective motions were very different in scale from the motions which we associate with modern plate tectonic phenomena. The ancient motions resulted in a distinctive surface expression. Many lines of evidence suggest that the Earth is cooling by heat and mass transfer which is reflected in magmatic phenomena at or near the surface. Evidence of convective mixing in all regions of the deep Earth is accumulating. Today, the major sites of mass and energy transfer are at the ocean ridges where new crust is formed. This motion towards the surface is balanced by the return of crustal material at the subduction zones. There are few sites of crust formation. They are all elongated, with linear dimensions comparable with the circumference of the Earth, and they are widely spread.

Geological maps of the crust of more than 3×10^9 yr ago indicate a totally different tectonic-magmatic situation. Such regions are well documented from southern Africa and Greenland¹. The sedimentary and igneous rock types in these regions have, in general, modern counterparts, and they provide evidence of the existence of an extensive hydrosphere. The rocks of the granite family, however, dominate 80–90% of the geologic maps and there are no significant areas of ophiolitic rocks; no analogues of the modern ocean crust². Metamorphic rocks of the granulite facies are very common and are often associated with anorthosite complexes³. Metamorphic events reflect steep thermal gradients. Extensive linear features are rare and volcanic-sedimentary basins involve complex mixtures of volcanic types from rhyolites and andesites to basalts and komatiites. This is not a modern situation.

A model for the early crust must take account of three concepts. Runcorn⁴ has summarised evidence which indicates that convective motions have been quantised through time. The evidence from age distribution patterns is compelling. Elder⁵ has indicated how the thermal structure of the Earth is appropriate to a body cooling from the surface and in a state of vigorous convection. The models involve a rather thin surface region with a steep thermal gradient followed by a deep, essentially isothermal region. Where the gradient changes rapidly, there is a layer of high thermal turbulence with large fluctuations of temperature (a possible low velocity or melting region). With time, this region will sink into the cooling body and the amplitude of fluctuations will decay. Finally, Fyfe and Leonardos⁶ point out that basalt will not continuously penetrate a fusible granitic layer; fusion of the granite, will form a viscous low density layer, and this will turn off injection or encapsulate the heavier magma. I use the term encapsulate in the sense in which it is used in the ceramic or metallurgical industries to describe the situation in which a heavy material is protected from the environment by a lighter, viscous, glassy layer.

The Archaean model proposed here is in part a result of an attempt to explain the map and synthesis proposed for the Rhodesian craton⁷ which is similar to many cratonic regions of a comparable age. Before any quantum jump

in the convective pattern⁴, the dynamic structure could be as shown in Fig. 1. A continuous, thin, highly radioactive, easily fusible granitic layer, 5–10 km thick (Fig. 1a), overlies a high-level melting zone in the upper mantle (Fig. 1b). In the melting zone partial melting occurs on a large scale, and various products of melting result from a high level of thermal turbulence. Convection cells in the mantle have a short wavelength pattern (about 100 km) as is implied by the separation of basins in Rhodesia⁷. Basalt penetration of the crust (Fig. 1c) is sporadic and is terminated by crustal fusion and the formation of granitic domes (Fig. 1d). Basalt flow is concentrated beneath the encapsulating acid layer. The basalt tends to underplate the acid crust and fractionates to produce anorthosite complexes which themselves will be associated with granulites, the crustal fusion residue (Fig. 1e). Above regions of return flow where the crust is thinned, volcanic rocks may be injected; basalts, rhyolites, or, where mixing occurs, andesites (Fig. 1f, g, h). During periods of maximum thermal turbulence and high partial fusion, the ultramafic komatiites may erupt.

The situation after the convective pattern changes might represent that pertaining only for a short time before the change. The quantum jumps are considered to be towards larger cells (compare with ref. 4). In the model of early patterns, heat-mass transfer in the upper layers involves a two-storey motion, with basic magmas adding heat to the acid layer which itself overturns by magmatic processes. Erratic convective motion could lead to thrusting of the mini-continent, which would produce the type of tectonics frequently observed when basement rocks are exposed.

It is proposed here that modern ophiolitic plate tectonics began only after the thermal regime of the acid crust allowed stable thickening to the point where granitic materials did not cover most of the earth. This idea does not conflict with palaeomagnetic results discussed by Piper *et al.*⁸ and perhaps provides the "supplement to plate theory of orogenesis" discussed in their work. It could be predicted that future convective patterns will lead to a further thickening of acid materials and a more extensive covering the basaltic crust.

W. S. FYFE

University of Western Ontario,
London, Canada

Received February 11, 1974.

¹ Sutton, J., and Windley, B. F., *Phil. Trans. R. Soc.*, **A273**, 315 (1973).

² Church, W. R., *Publication of the Earth Sciences Branch, Department of Energy, Mines and Resources, Canada*, **42**, 71 (1972).

³ Fyfe, W. S., *Phil. Trans. R. Soc.*, **A273**, 457 (1973).

⁴ Runcorn, S. K., *Phil. Trans. R. Soc.*, **A258**, 228 (1965).

⁵ Elder, J. W., *J. Earth Sci., Leeds*, **8**, 287 (1972).

⁶ Fyfe, W. S., and Leonardos, O. H. jun., *Nature*, **244**, 501 (1973).

⁷ MacGregor, A. M., *Proc. geol. Soc. S. Afr.*, **54**, 27 (1951).

⁸ Piper, J. D. A., Briden, J. C., and Lomax, K., *Nature*, **245**, 244 (1973).

Electrons and the emission of soot from flames

AN increasing body of evidence suggests that soot formation is associated with ionisation in flames and may be controlled electrically. Work in which sooting flames are subjected to large electric fields¹⁻³ shows that all soot particles are charged (at least in the presence of an applied field) and can therefore be manipulated by fields. This applies not only to fully formed particles but also to their growth and burning up in flames. Thus by applying fields so as to remove particles rapidly from the zone in which they are formed, their size as well as the total yield is greatly reduced. The former is ascribed to the decreasing residence times and the latter to the removal of surface on which growth can occur. Conversely, very large particles can be produced by holding them stationary against the gas flow by means of a field and thereby increasing their residence times. Also, any appreciable field intensity removes the charged nuclei on which carbon particles tend to grow³.

Many practical systems are not amenable to the application of large electric fields and a great many research projects have concerned themselves with the effect of additives (refs 4-8 and E. M. Bulewicz, D. G. Evans, and P. J. Padley, personal communication). The most effective additives have generally proved to be substances which have low ionisation potentials or form particles of low work functions. Thus the alkali metals, when introduced to the extent of parts per million, alter the 'structure' of carbon black and the effectiveness of the additive in breaking long chains is in the inverse order of its ionisation potential. Although the order of effectiveness within each group of elements is determined by the ionisation potential, this does not apply across groups, the alkaline earths, for example, differing from the alkali metals, barium additives which produce BaOH^+ as the positive ion being the most effective. Also, the same additive can produce suppression of soot formation, or its promotion, depending on the amount and the position in which it is introduced. Where the position of addition is not localised, the pattern is that small amounts produce promotion whilst large amounts produce suppression with a crossover point of zero effect in between. When the additive is introduced locally—on a wire, say—insertion at the base of the flame produces suppression; introduction higher up results in an increase of soot produced⁶.

Chemical—as an alternative to electrical—effects have been considered responsible for some of these observations⁶. Since electronic structure is inevitably related to chemistry, the issue is most likely to be resolved by the application of electric fields. Different groups, however, have used either additives, or applied fields—not both simultaneously. We now propose a hypothesis based on free electrons and then test it by the combination of the two techniques. Although the results bear out the hypothesis and, furthermore, offer a novel practical method for controlling soot emission, this does not, of course, constitute a proof that chemical effects could not also be important.

Most of this work has been carried out in diffusion flames—in which the reactants are initially separate the maximum temperature and the main reaction zone occurring close to the stoichiometric contour. This is also close to the surface along which ionisation will peak, being a strong function of temperature. It is not, however, the region of carbon formation, since conditions for pyrolysis are most favourable on the fuel side, where there is a lack of oxygen. Thus if ionisation is to play a major part in soot formation, the diffusion coefficient of the charge carrier must be a most important parameter. Of the particles involved, it is the electron that has by far the largest diffusion co-

efficient and it is known that the negative charge carrier in the high temperature zones of flames is an electron². We therefore suppose, as an initial hypothesis, that the effect of additives is entirely due to the free electrons which they

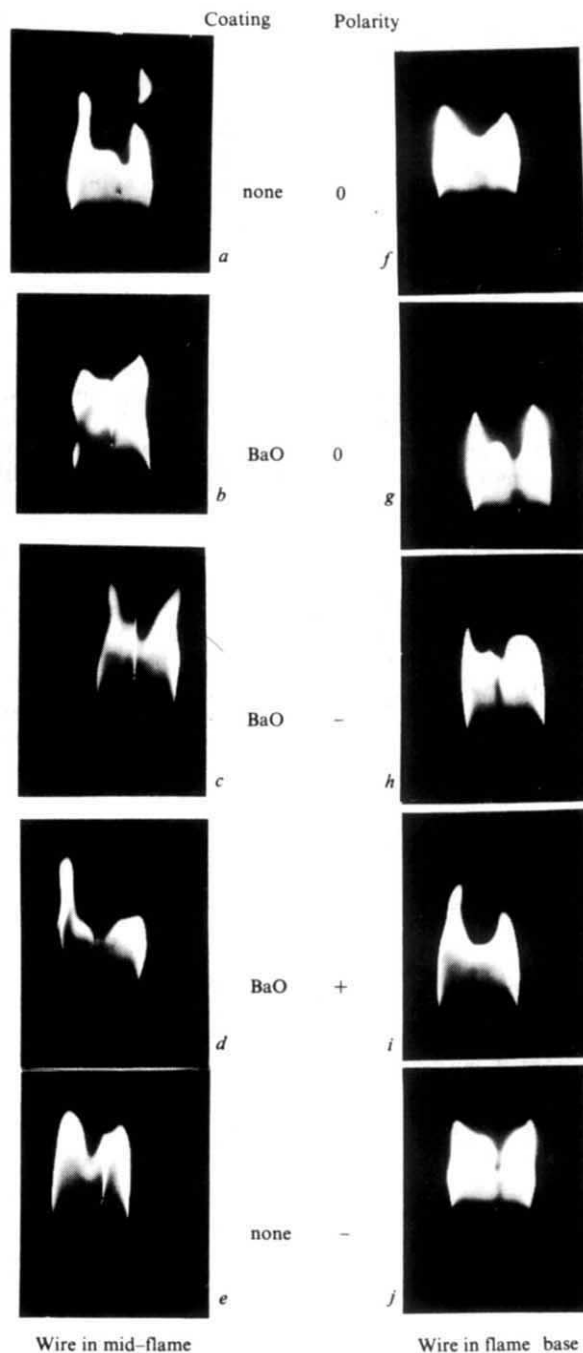


Fig. 1 Effect of coating and polarity on soot formation above wires.

produce. We shall then attempt to account for all previous experimental observations in terms of such a hypothesis and, if that is successful, design some further crucial experiments.

Although the chief role of an influx of additional electrons into the pyrolysis zone is likely to be that of neutralising positive charges, this can have two diametrically opposed consequences. Where all or most of the particles¹⁻³, or

chains of particles, are positively charged, neutralisation of positive charges at their extremities would stop them repelling one another and thereby remove the obstacle to agglomeration. Agglomerates of appreciable size are much less likely to burn away during their subsequent passage through the high temperature zones in the presence of oxygen. When only a fraction of the chains is charged, on the other hand, agglomeration occurs by dipole action, the rate reaching a maximum when the fraction charged is a half. For smaller proportions of charged particles, electrons will therefore decrease agglomeration rates.

This proposition accords extremely well with the observed facts. Large electron concentrations will eventually always inhibit agglomeration and thereby suppress soot formation. Electron concentrations insufficient to neutralise a very large proportion of particles under conditions where the majority of particles is charged will act mostly as a 'glue' in encouraging small particles to aggregate and thereby increase the mass of soot escaping.

The dependence on the mobility of the positive ion also falls into place, although the role attributed to it is diametrically opposed to what has been supposed hitherto. In terms of the above hypothesis, the positive ion acts chiefly through recombining with the electrons it meets away from the high temperature zone (it is of course much more likely to encounter an electron than a carbon particle). The larger the ion the smaller its excursion.

Although this tentative hypothesis thus accounts for all the experimental observations, a much more direct test would be one in which the additive would be discouraged from migrating by being held on a wire at a relatively low temperature, whilst the charges emanating from it could be controlled by an electric potential applied to the wire.

Nichrome wires coated with barium oxide and uncoated wires of diameter 0.2 mm were inserted at various heights into diffusion flames of propane diluted with argon, burning with air. The flames consisted of single, approximately flat, faces, which were contrived very simply by attaching a 'fish-tail' nozzle to the fuel burner tube and blowing off the second flame face by an adjacent argon stream emerging from a similar nozzle. The results are shown in the photographs of Fig. 1 and the current-voltage characteristics of Fig. 2.

The results are in exact accord with the predictions of the hypothesis, in that it is the electron which produces effects characteristic of the additive. At heights where the additive promotes soot formation (Fig. 1*b* as compared with Fig. 1*a*) application of a negative potential, encouraging the emission of electrons, greatly increases the effect (Fig. 1*c*) and the application of a sufficient positive potential prevents it altogether (Fig. 1*d*). It transpired from the current-voltage characteristics that positive potentials not only prevented electron emission but led to the emission of positive ions (Fig. 2) and some ionic wind effects came into evidence at higher currents. The absence of soot formation in the case of positive ion emission casts doubt on previous suppositions that positive inorganic ions themselves act as nuclei; they do of course mop up electrons.

We tried, unsuccessfully, to explain these effects in terms of what is already known about the effects of electric fields on carbon formation. According to this, the negatively charged wire would be expected to collect the positively charged carbon particles—and this is indeed precisely what happens in the case of the uncoated wire (Fig. 1*e*)—rather than to increase soot formation. Once a layer of carbon is built up on the wire, of course, electron emission sets in.

Figure 1(*f-j*) illustrates the effects observed when wires are inserted into zones in which suppression is produced by the additive in the absence of an applied potential (Fig. 1*g*). Although these are the reverse of the pattern of Fig. 1*a-e* they also can be summarised by the observation that the behaviour associated with additives is manifested by free electrons. It is accentuated when the applied field pro-

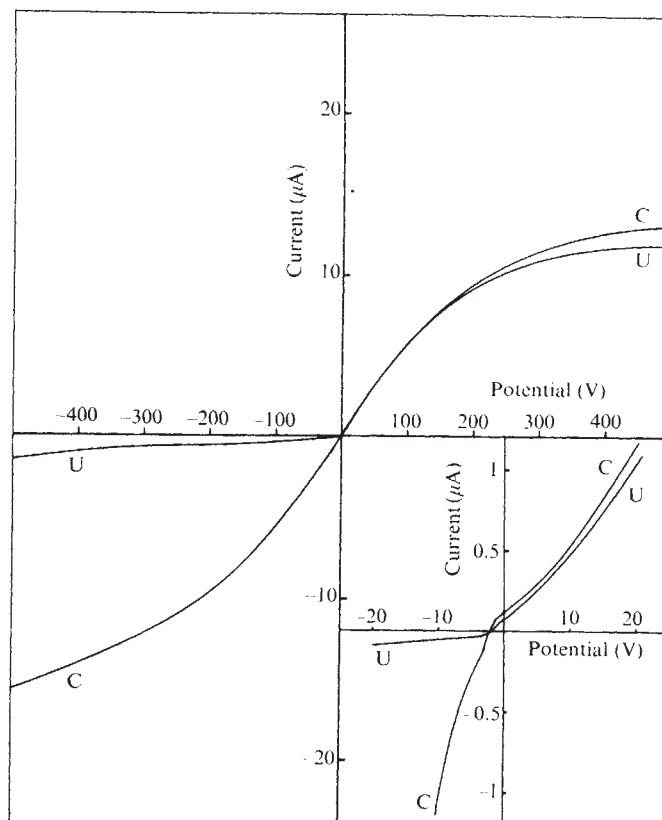


Fig. 2 Current/potential characteristics of the wire with respect to the burner, of coated (C) and uncoated (U) wires in mid-flame.

motes electron emission and tends to reverse when electron emission is prevented by positive polarity of the support.

We do not suggest that the entire subject of sooting in flames can be accounted for in terms of free electrons and we think it very likely that the underlying chemical processes retain the importance that has been attributed to them. But it does seem possible to account for the behaviour of ionising additives entirely in terms of the electrons they produce as regards both suppression and promotion, the effect of the amount of additive, the site of its introduction and the effect of ion size.

What is probably much more important is that we can produce all the observed effects by controlling the emission of free charges electrically, using coated surfaces maintained hot by the flame, and connected to a source of electrical potential—without introducing additives into the fuel and, thereafter, into the atmosphere by way of exhaust gases. The power that would be involved in injecting electrons in this manner would, of course, be quite small, 1 mA being sufficient to provide 6×10^{15} particles s^{-1} .

We thank Drs Bulewicz and Padley for comments and Esso Research, Abingdon, for financial support to one of us (R.J.B.).

R. J. BOWSER
F. J. WEINBERG

Department of Chemical Engineering
and Chemical Technology,
Imperial College, London, SW7, UK

Received January 29, revised March 7, 1974.

¹ Place, E. R., and Weinberg, F. J., *Proc. R. Soc.*, **A289**, 192–205 (1965).

² Lawton, J., and Weinberg, F. J., *Electrical Aspects of Combustion* (Clarendon Press, Oxford 1969).

³ Mayo, P. J., and Weinberg, F. J., *Proc. R. Soc.*, **A319**, 351–371 (1970).

- ⁴ Addecott, K. S. B., and Nutt, C. W., *Am. Chem. Soc. Div. Petrochem.*, **14** (4), A69-80 (1969).
⁵ Cotton, D. H., Friswell, N. J., and Jenkins, D. R., *Combustion and Flame*, **17**, 87-98 (1971).
⁶ Salooja, K. C., *Nature*, **240**, 350-351 (1972).
⁷ Salooja, K. C., *Combustion Institute European Symposium* (edit. by Weinberg, F. J.) 400-405 (Academic Press, London, 1973).
⁸ Feugier, A., *ibid.*, 406-411.

Radicals in irradiated ethyl iodide and related compounds

IN studies of the radiolysis of various alkyl halides, particular attention has been focused on a multiline electron spin resonance (ESR) spectrum obtained from ethyl iodide at 77 K (ref. 1). Despite the use of a wide range of isotopically enriched materials, however, there has been no satisfactory interpretation of these complex ESR spectra and this remains one of the unsolved problems of ESR spectroscopy².

The spectrum for normal $\text{CH}_3\text{CH}_2\text{I}$ comprises sets of 'parallel' and 'perpendicular' type features covering a total range of about 1,000 gauss which seem, in the outer regions of the spectra, to comprise sextets with $a \approx 22$ gauss. These are centred close to the free-spin g value (see Figures in ref. 1).

Studies of other alkyl halides³⁻⁵, indicate that the most likely candidates are the α - or β -iodo alkyl radicals (Fig. 1, I and II). Radical I is, however, expected to exhibit a very marked g -value variation which would lead to considerable spectral asymmetry,

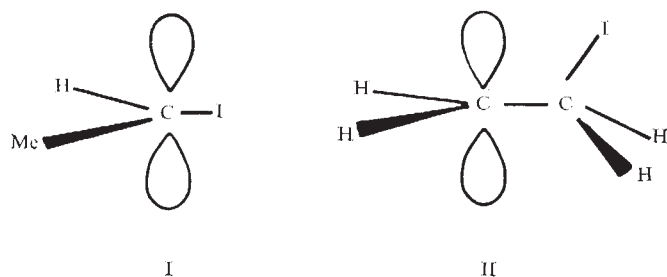


Fig. 1

with strong features in the region of low-field, and even if these radicals were librating or rotating the resulting g_{av} value would be > 2.0023 . This is not observed, and indeed, the spectra are completely different from that obtained from irradiated α -iodo acetamide, which we assign to $\text{HCl}(\text{CONH}_2)$ radicals (G. W. Neilson, and S. P. Mishra, unpublished results).

Again, the β -iodo radical, II, is expected to have a far larger hyperfine coupling to ^{127}I than is possible for the ethyl iodide radical, and the α -proton coupling should give rise to a triplet having $a \leq 20$ gauss. (Coupling to the two β -protons should be small.)

Thus I and II can be firmly rejected. The radical $\text{CH}_3\text{CH}_2\text{---I}^\cdot$ is, however, a likely candidate. Methyl bromide in a methyl cyanide matrix gives a species $\text{CH}_3\text{---Br}^\cdot$, which has the normal magnetic properties for methyl radicals, but with each feature split into a pair of quartets by hyperfine coupling to ^{79}Br and ^{81}Br (ref. 6). We have extended this work to include methyl iodide, which again gave a spectrum characteristic of methyl, with a weak hyperfine coupling to iodine⁷ (Table 1).

We find that the spectra published by Willard *et al.*¹, and ourselves for normal $\text{CH}_3\text{CH}_2\text{I}$ can be quite satisfactorily analysed on this hypothesis, using the parameters given in Table 1. In particular, the 22 gauss sextets have their $\pm 5/2$ components broadened, as is usually the case for ethyl radicals in rigid media, and therefore could be mistaken for quartets (1:3:3:1), thus closely resembling the features for $\text{CH}_3\text{CD}_2\text{---I}^\cdot$, as noted by Willard *et al.*¹.

Comparable species were not obtained from methyl iodide or from solutions of ethyl iodide in CD_3OD . A very similar

Table 1 ESR parameters for $\text{CH}_3\text{CH}_2\text{---I}^\cdot$ and related anions

Medium	Radical	Hyperfine coupling (gauss) *, †	
		^1H	^{127}I
$\text{CD}_3\text{OD}^\ddagger$	$\text{CH}_3\text{---I}^\cdot$	21 ()78	(⊥)56
$\text{C}_2\text{H}_5\text{I}$	$\text{CH}_3\text{CH}_2\text{---I}^\cdot$	22 ()168	(⊥)126
Me_3CI	$\text{Me}_3\text{C---I}^\cdot$	22 ()>53.5	(⊥)53.5

*, 1 gauss = 10^{-4} T;

†, all g values close to 2.0023;

‡, ref. 6.

species, $(\text{Me})_3\text{C}^\cdot\text{---I}^\cdot$, was, however, obtained from *t*-butyl iodide (Table 1).

We conclude that the process involved is



in which the radical, $\text{R}^\cdot$, has its normal planar geometry, but, under favourable circumstances, exhibits a very weak charge-transfer interaction with iodide. We suggest that the occurrence of this interaction, and its magnitude, is governed primarily by the crystal structure of the host compound and therefore by the mobility of the alkyl radical. The results for CD_3OD solution show that the interaction is lost when iodide can be solvated, even at 77 K.

A. R. LYONS

M. C. R. SYMONS

S. P. MISHRA

Department of Chemistry,
The University,
Leicester LE1 7RH, UK.

Received October 8, 1973.

¹ Egland, R. J., Ogren, P. J., and Willard, E. E., *J. phys. Chem.*, **75**, 467 (1971) and references therein.

² *Electron Spin Resonance*, Vol. 1 (edit. by Norman, R. O. C.) Chemical Society Specialist Report, 130 (London, 1973).

³ Kispert, L. D., and Myers, F., *J. chem. Phys.*, **56**, 2623 (1972).

⁴ Mishra, S. P., Neilson, G. W., and Symons, M. C. R., *J. Am. chem. Soc.*, **95**, 605 (1973).

⁵ Lyons, A. R., and Symons, M. C. R., *J. Am. chem. Soc.*, **93**, 7330 (1971).

⁶ Sprague, E. D., and Williams, F., *J. chem. Phys.*, **54**, 5425 (1971).

⁷ Mishra, S. P., and Symons, M. C. R., *J. chem. Soc., Perkin II*, 391 (1973).

Lyoluminescence of trehalose dihydrate

LYOLUMINESCENCE of trehalose.2H₂O in water has been observed over a wide range of radiation doses. It has been possible to cover linearly the range from 10 rad up to 150 krad. With some samples the lower limit was 100 mrad.

The emission of light when irradiated saccharides are dissolved in water has been described^{1,2}. Saccharides were chosen because of their good solubility in water and because they are quite good soft tissue equivalent materials. Free radicals which formed during irradiation of the solid probably cause the light emission when they are set free to react in solution³.

There is evidence from our experiments that the diffusion controlled reactions involved in the process of light emission are of the type:



This is based on the several observations.

(1) The emission spectrum of all saccharides (for example, trehalose.2H₂O, glucose, mannose) has a maximum at approximately 4,900 Å as determined by interference filters. This coincides with the absorption peaks of the S→T transition of $(\text{O}_2)_2$ associate⁴.

(2) When irradiated saccharides are annealed at different temperatures, they tend to exhibit more lyoluminescence. This is a result of the temperature-assisted diffusion of O_2 during annealing.

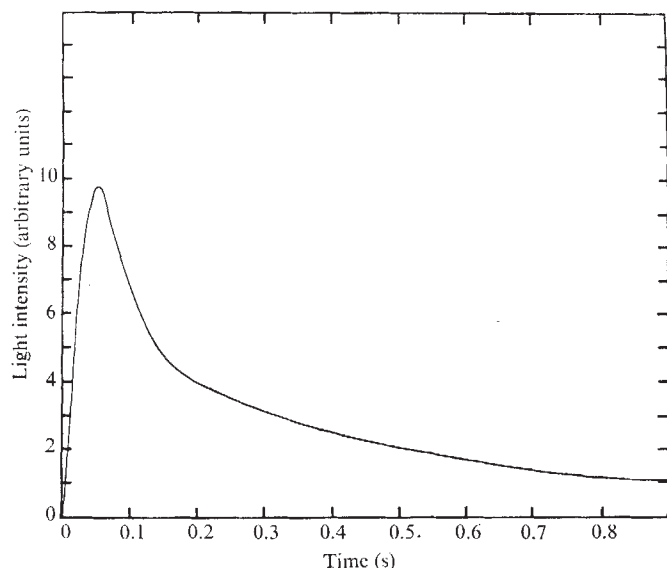


Fig. 1 Lyoluminescence decay curve of trehalose.2H₂O in water.

(3) The addition of $\approx 10^{-4}$ M concentration of Cu⁺, Cu²⁺, Mn O₄⁻ and Fe(CN)³⁻ quenches the light to 31%, 14%, 4% and 1.5%, respectively, of the value for pure water. Addition of fluorescein has no effect, whereas water saturated with O₂ enhances the light emission. The quenching effect of these ions is because of the tendency of radicals to react preferentially with them instead of with oxygen. The failure of fluorescein to enhance the light, suggests that no energy transfer occurs between the produced R-R and fluorescein molecules.

(4) Reaction (2) has to compete with reaction (1). At higher concentrations of radicals, that is, at high doses, reaction (1) becomes more frequent as the distance between radicals decreases. This explains the saturation and the decrease in the light yield over 150 krad, which is common for all saccharides (Fig. 3). The actual saturation level in solid saccharides, as determined by ESR, is over 100 Mrad (ref. 5).

When luminol solution was used instead of water the enhancement of light emission was a million times in the case of glucose and twenty times for trehalose.2H₂O. The light emission accompanying the oxidation of luminol strongly suggests that RO₂, with its oxidising properties, is involved in the process.

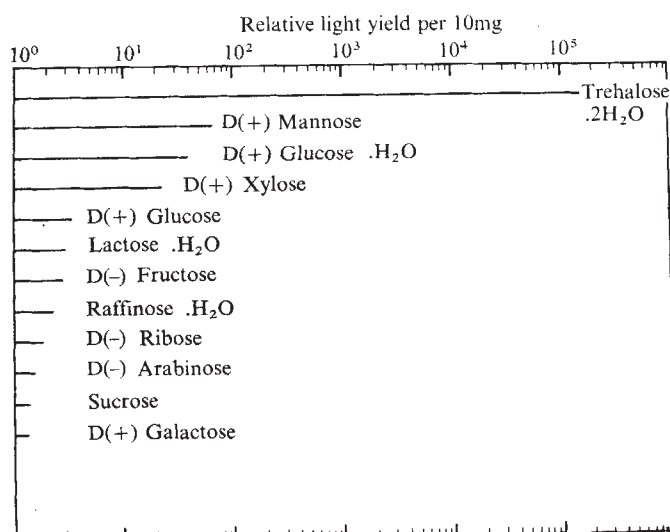


Fig. 2 Lyoluminescence response of saccharides to radiation. All given the same dose (67 krad) using a ⁶⁰Co γ source.

Figure 1 shows the lyoluminescence decay curve of trehalose.2H₂O in water. The light which can be seen easily with the naked eye, was integrated for a period of 3 s, which adequately covers the light output. The relative lyoluminescent sensitivity of various saccharides is presented in Figure 2.

The relationship between the radiation dose (using a ⁶⁰Co γ source) and the integrated light intensity is shown in Figure 3. The curve is linear between 100 mrad and 150 krad. The lower limit is mostly controlled by the dark current of the photomultiplier tube and can be improved by one order of magnitude if a cooled tube is used. It also depends on the lyoluminescence response of trehalose which varies for samples of different origin.

The lyoluminescence stability of trehalose.2H₂O after irradiation was revealed by the isothermal annealing after

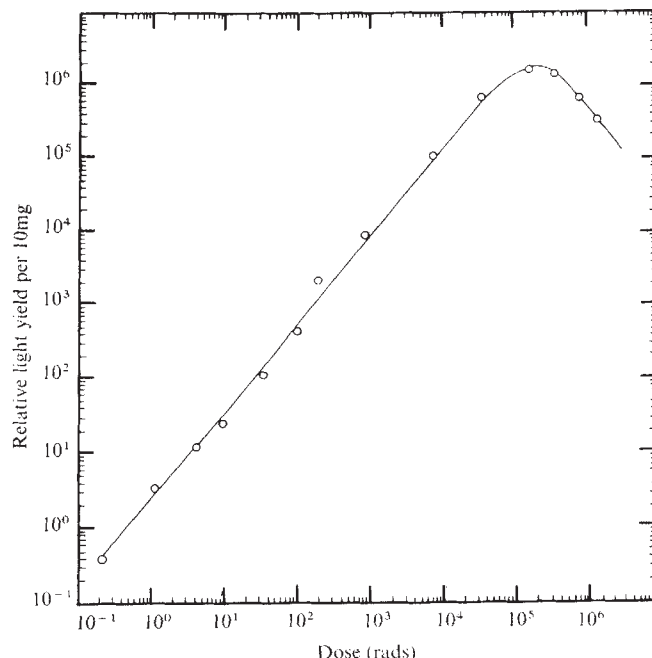


Fig. 3 Light yield against radiation dose for trehalose.2H₂O dissolved in water.

irradiation. A sample heated to 80° C for 2 h showed no change in the light output and heating to 60° C for 60 h caused a decrease of only 18%. These results are consistent with the measurements of Löfroth *et al.*⁶, who found that irradiated trehalose.2H₂O loses only 10% of its ESR signal after storage for six months.

We thank Professor J. H. Fremlin for guidance and advice. This work was supported by the Science Research Council.

N. A. ATARI*
K. V. ETtinger*

Department of Physics,
Chancellors Court,
The University of Birmingham,
P.O. Box 363,
Birmingham, B15 2TT, UK

Received August 10, 1973.

*Present address: Department of Medical Physics, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD, UK.

¹ Atari, N. A., Ettinger, K. V. and Fremlin, J. H., *Radiat Effects*, **17**, 45 (1973).

² Atari, N. A., and Ettinger, K. V., *Radiat Effects*, **20**, 135 (1973).

³ Ahnström G., and Ehrenstein, G., *Acta chem. scand.*, **13**, 199 (1959).

⁴ Stauff, J., Schmidkunz, H., and Hartmann, G., *Nature*, **198**, 281 (1963).

⁵ Löfroth, G., *Acta chem. scand.*, **21**, 1997 (1967).

⁶ Löfroth, G., Ehrenberg, L., and Ehrenberg, A., *Proceedings of the Fifth International Symposium on Free Radicals, Stockholm, 1961* (Almqvist and Wiksell, Stockholm, 1962).

BIOLOGICAL SCIENCES

SV40 nucleoprotein complex activity
unwinds superhelical turns in SV40 DNA

THE replication of closed circular superhelical DNA of simian virus 40 (SV40) has been shown to proceed through replicative intermediates that are composed of newly synthesised linear polynucleotide chains hydrogen bonded to closed circular template (parental) strands¹⁻³. To replicate these molecules in a semiconservative fashion a swivel, about the phosphodiester backbone of the template strand of DNA, required during polynucleotide chain propagation^{2,3}. Such a swivel could be obtained by a nuclease which introduces a single-stranded break in the template strand, thereby allowing free rotation about the phosphodiester bond opposite the nick and relaxing the superhelical turns in the DNA molecule. This type of single-stranded break must, however, be transient and rapidly ligated closed in the cell because breaks in the template strands of replicative intermediates have not been detected¹⁻³. Alternatively, an activity has been described in *E. coli*^{4,5} and mouse cells⁶, that can relax superhelical turns in closed circular DNAs without introducing either single or double-stranded chain scissions in the DNA product of this reaction. This 'untwisting activity' is also a potential candidate for the swivel required during SV40 DNA replication.

The replicative intermediates of SV40 DNA have most often been characterised^{1,2,7} after lysing SV40-infected cells with sodium dodecyl sulfate (SDS) in the presence of 1 M NaCl¹. This results in the isolation of viral DNA molecules that contain little or no protein associated with the DNA⁷. When polyoma⁹ or SV40-infected^{10,11} cells are lysed instead with the nonionic detergent Triton X-100 in the presence of 0.2 M NaCl, all the viral DNA is recovered in a rapidly sedimenting nucleoprotein complex (44-53S). This complex contains both mature viral DNA as well as the SV40 replicative intermediates^{10,13}, associated with histone-like proteins and at least two of the viral coat proteins coded for by the *tsB8* and *tsB11* loci of SV40^{11,12}. The complex containing replicative intermediates sediments faster than that composed solely of mature viral DNA^{10,13}.

Here we report that the SV40 nucleoprotein complex has associated with it an untwisting activity that reduces the number of superhelical turns in exogenously added SV40 DNA. The underwound product of this reaction remains closed and circular as detected in the ethidium bromide (EtBr)-caesium chloride (CsCl) dye-density gradient. This activity has been shown to be associated with the nucleoprotein complex that contains either mature or replicative intermediates of viral DNA.

SV40-infected African Green Monkey Kidney cells were labelled with ³H-thymidine for 20 h, starting 16 h post-infection⁷. The SV40 nucleoprotein complex was isolated as described in Fig. 1. After concentrating the preparation and dialysis, the complex was incubated at 37°C for 2 h in the presence or absence of exogenous purified closed circular superhelical (Form I) SV40 DNA⁷. At the end of this incubation period, ³²P-labelled Form I SV40 DNA was added (at 0°C) to the sample incubated in the absence of exogenous SV40 DNA. Each of these reaction mixtures was then analysed in an EtBr-CsCl equilibrium gradient to determine the amount of Form I and II DNAs present in each sample and to monitor the superhelical density of the endogenous DNA (³H-labelled) in the nucleoprotein complex and exogenously added ³²P-labelled DNA.

The results of the EtBr-CsCl gradients are presented in Fig. 1. Two conclusions can be drawn from these data: First, incubation at 37°C for 2 h of 50S complex alone or

this complex with exogenously added Form I SV40 DNA resulted in no detectable endonuclease activity that generated relaxed (Form II or III) SV40 DNA molecules (Table I) under these assay conditions. When the 50S nucleoprotein complex was incubated with ³²P-labelled exogenous SV40 Form I DNA, however, the superhelical density of this DNA was decreased as seen by a shift of the ³²P-labelled viral DNA to a heavier density position in dye-density gradients (Fig. 1b). The superhelical density of Form I DNA in the complex (endogenous; ³H-labelled) may also show a small reduction in the number of superhelical turns (Fig. 1a, b).

To determine if this untwisting activity was preferentially associated with the nucleoprotein complex containing mature DNA or replicating intermediates, the experiment described above was repeated. A second neutral sucrose gradient was used to separate the faster sedimenting component of the nucleoprotein complex that contains mainly replicating DNA from the complex containing mostly mature viral DNA. The untwisting activity was found to be associated with both species of this complex.

These experiments demonstrate the presence of an untwisting activity, associated with 50S nucleoprotein complex, that could act as a swivel during replication of

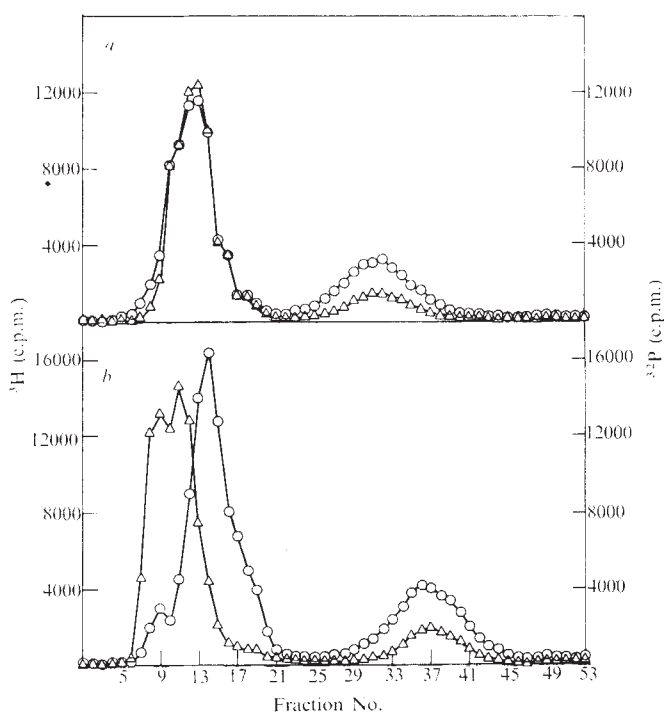


Fig. 1 EtBr-CsCl equilibrium gradients of SV40 DNA incubated with the SV40 nucleoprotein complex. SV40-infected AGMK cells were labelled with ³H-thymidine 16-36 h after infections. The cells were lysed as described previously and the 50S complex was isolated from a sucrose gradient (ref. 9 and A. S., R. Hancock, and A. J. L., unpublished). The complex was then concentrated (5-8 fold) by dialysis against glycerol followed by dialysis against 10 mM Tris buffer (pH 7.4), 0.2 M NaCl, 4 mM EDTA and 1 mM β -mercaptoethanol. The ³H-labelled (thymidine) nucleoprotein complex was incubated at 37°C for 2 h with or without exogenously added ³²P-labelled purified Form I SV40 DNA (ref. 7). The reaction mixture contained 0.5 μ g Form I viral DNA (1.5×10^5 c.p.m. μ g⁻¹), 10 mM Tris buffer (pH 7.9), 0.1 M NaCl, 2 mM EDTA. The reaction was stopped by adding 1 M NaCl and 1.598 g ml⁻¹ of CsCl ($\eta = 1.3900$). 100 μ g ml⁻¹ of EtBr was added (final concentration) and this mixture (6 ml total) was spun to equilibrium at 32,000 r.p.m. for 90 h in a fixed angle 50 rotor. Samples were collected and counted as described previously⁷. O, ³H c.p.m. endogenous complex SV40 DNA; Δ , ³²P c.p.m. exogenously added SV40 DNA.

Table 1 Test for nuclease activity in the 50S complex

Procedure	Endogenous DNA		Exogenous DNA	
	Form I	Form II	Form I	Form II
No incubation	90%	10%	76%	24%
Incubation: 2 h at 37°C (50S complex alone)	88%	12%	—	—
Incubation: 2 h 37°C (50S complex plus exogenous DNA)	94%	6%	72%	28%

The experimental details are presented in the legend to Fig. 1. The percentage of Form I and II DNA observed after each incubation produce was calculated from the experimental points in Fig. 1.

superhelical SV40 DNA. The untwisting activity cosediments with the 50S complex upon repeated centrifugation, and is not found sedimenting at the 50S position of a sucrose gradient when uninfected cells are lysed under similar conditions, but it is not clear whether this activity is associated with nucleoprotein complex *in vivo*. Experiments are in progress to characterise conditions where the untwisting activity will act as efficiently upon endogenous viral DNA in the nucleoprotein complex as on exogenously added purified Form I DNA. When such conditions are determined, the nucleoprotein complexes containing replicating viral DNA would then become useful templates for an *in vitro* DNA replication system.

ARUP SEN
ARNOLD J. LEVINE

Department of Biochemical Sciences,
Princeton University,
Princeton, New Jersey 08540

Received January 4, 1974.

- ¹ Jaenisch, R., Mayer, A., and Levine, A. J., *Nature new Biol.*, **239**, 235 (1972).
- ² Sebring, E. D., Kelly, T. J., Thoren, M. M., and Selzman, N. P., *J. Virol.*, **8**, 478 (1971).
- ³ Mayer, A., and Levine, A. J., *Virology*, **50**, 328 (1972).
- ⁴ Wang, J. C., *J. molec. Biol.*, **43**, 236 (1969).
- ⁵ Wang, J. C., *J. molec. Biol.*, **55**, 523 (1971).
- ⁶ Champoux, J. J., and Dulbecco, R., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 143 (1972).
- ⁷ Levine, A. J., Kang, H. S., and Billheimer, F. E., *J. molec. Biol.*, **50**, 549 (1970).
- ⁸ Hirt, B., *J. molec. Biol.*, **26**, 365 (1970).
- ⁹ Green, M. H., Miller, H. J., and Hendler, S., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1032 (1971).
- ¹⁰ Eason, M., and White, R., *J. Virol.*, **8**, 363 (1971).
- ¹¹ Sen, A., Hancock, R., Levine, A. J., *Virology* (in press).
- ¹² Tentmayer, P., and Ozer, H. L., *Virol.*, **9**, 516 (1971).
- ¹³ Mayer, A., thesis, Princeton Univ., (1972).

Enzymatic aziridine synthesis from β aminoalcohols—a new example of endogenous carcinogen formation

BETA aminoalcohols are not only used as drugs, such as ephedrine, chloramphenicol or pronethalol, but also occur naturally in animals and man, as adrenaline and noradrenaline, for example. It would be interesting to know whether these β aminoalcohols can be metabolised to aziridines, a class of carcinogenic compounds. Howe¹ described the carcinogenicity of pronethalol (I) and supposed that the aziridine (II) could be the ultimate carcinogen (Fig. 1).

In vitro formation of aziridines from β aminoalcohols only occurs through a less stable intermediate, for example by the sulphate ester in the Wenker synthesis². On the other hand, the enzymatic formation of sulphate esters is catalysed by various sulphotransferases and requires phosphoadenosinephosphosulphate (PAPS) as a sulphate donor³. For practical purposes PAPS can be substituted by a mixture of ATP and SO_4^{2-} , which is transformed partially into PAPS by enzymes, contained

in the supernatant fraction of liver homogenates⁴. It should be noted, however, that the carcinogenic 2-acetylaminofluorene is also metabolised through a sulphate ester to the ultimate carcinogen⁵. We supposed that the hydroxyl group of β aminoalcohols also could react enzymatically in special conditions to form O sulphates. These sulphates can then be transformed into aziridines in a slightly alkaline medium. To confirm this hypothesis we investigated the enzymatic formation of 1, 2-dimethyl-2-phenylaziridine⁶ (III) from the β aminoalcohol⁶ (IV), an isomer of ephedrine (Fig. 1):

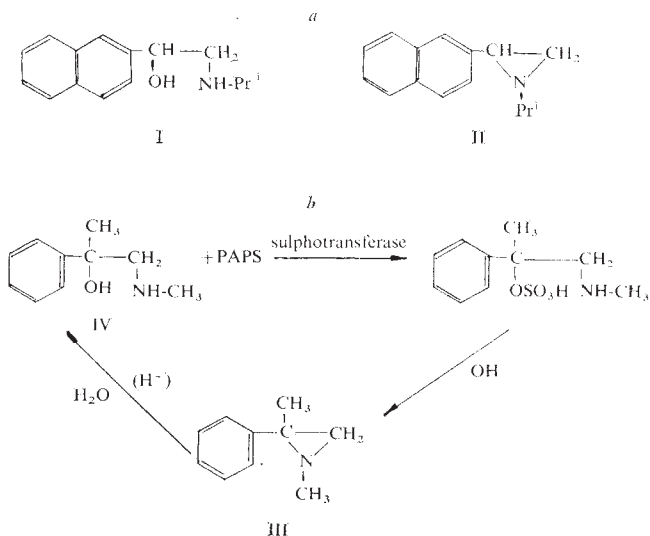


Fig. 1 a, Pronethalol (I) and its aziridine (II); b, enzymatic formation of 1, 2-dimethyl, 2-phenylaziridine from the β aminoalcohol.

A liver homogenate of a female Hauben rat (20% in 0.1 M potassium phosphate buffer, pH 7) was prepared by means of a Potter homogeniser and centrifuged at 100,000g for 1 h. To 5 ml of the supernatant, 75 μmol IV, dissolved in 0.05 ml ethanol, 50 μmol ATP, 50 μmol K_2SO_4 and 50 μmol MgCl_2 were added. After 2.5 h incubation at 37°C the reaction was terminated by the addition of 10 ml of ethanol and the precipitate removed by centrifugation. The supernatant was adjusted to approximately pH 8 by 1N NaOH and incubated at 37°C for 1 h. After evaporating to 5 ml under reduced pressure, the alkaline solution was extracted with ether. Basic compounds were separated from the ether solution by extraction with 2N H_2SO_4 . The acid solution was alkalinised immediately to avoid opening the ring of the aziridine formed. Repeated extraction with ether and evaporation to about 0.5 ml yielded a concentrate, which was investigated by means of TLC on silica gel. The solvent systems used were ethyl acetate-ammonia-methanol (85 : 5 : 10) and benzene-acetone (1 : 1), respectively.

For detecting aziridines, the colour reaction of 4-(4-nitrobenzyl)-pyridine perchlorate (NBP- ClO_4) with alkylating agents⁷ was modified. The plate was sprayed with 0.5% NBP- ClO_4 in 1, 2-dimethoxyethane, incubated at 100°C for 30 min and, after cooling, sprayed with 25% aqueous ammonia. The aziridine (III) appeared blue with R_f values of 0.80 and 0.32, respectively, identical with those of authentic samples in both systems. After standing overnight in 2N H_2SO_4 , III could not be detected in the extract, indicating ring-opening to IV. When SO_4^{2-} and ATP were not added to the reaction mixture, aziridine (III) was not formed. We therefore suppose that the aziridine is formed enzymatically from the β aminoalcohol according to the pathway shown in Fig. 1.

These investigations were made from the point of view that aziridines could be synthesised enzymatically not only from various drugs, but also from biogenic β aminoalcohols. This

aziridine synthesis might be one of the causes of tumours in animals and men¹.

Further investigations on the formation of aziridines from the above mentioned β aminoalcohols and on the carcinogenicity of these compounds are in progress.

We thank Professor E. Schmitz, Academy of Science of the GDR, for his support.

U. BICKER
W. FISCHER

Institute of Pathology,
Chemical Department,
Erfurt Medical Academy,
Nordhauser Strasse 74,
GDR 50 Erfurt.

Received January 29, 1974.

¹ Howe, R., *Nature*, **207**, 594 (1965).

² Wenker, H., *J. Am. chem. Soc.*, **57**, 2328 (1935).

³ Williams, R. T., in *Biogenesis of Natural Compounds*, second ed. (edit. by Bernfeld, P.) 589 (Pergamon Press, New York, 1967).

⁴ De Meio, R. H., and Lewycka, C., *Endocrinology*, **56**, 489 (1955).

⁵ De Baun, J. R., Miller, E. C., and Miller, J. A., *Cancer Res.*, **30**, 577 (1970).

⁶ Bicker, U., Dissertation, German Academy of Science, (Berlin, 1972).

⁷ Preussmann, R., Schneider, H., and Eppele, F., *Azrneimittel-Forsch.*, **19**, 1059 (1969).

Intragenic regulation of the synthesis of Φ X174 gene A proteins

ONLY one phage gene, A, has been shown to be necessary for the replication of Φ X174 double-stranded replicative form (RF) DNA¹⁻³. The host genes required have yet to be fully determined although one host mutation, *rep*⁻³, prevents progeny RF synthesis⁴. Dissection of the characteristics of gene A has shown that it is (a) *cis* acting⁵; (b) necessary for the detection of a *cis*-related viral strand specific discontinuity in the parental RF^{6,7}; (c) the region of the genome which contains the origin of DNA replication⁸, and (d) a gene from which two proteins, A and A' (molecular weights 35,000 and 60-65,000, respectively) are synthesised^{9,10}.

A model for the synthesis of two proteins from one gene is shown in Fig. 1. The synthesis of the smaller protein, A, is initiated internal to gene A. Amber mutants mapping from N14 to R7 synthesise only the A molecule, while amber mutants mapping from H90 to S29 do not synthesise either molecule⁹. The tryptic peptides of A' have been shown to include the tryptic peptides of A in addition to unshared peptides¹⁰.

While trying to understand how two proteins could be synthesised from one gene, we noticed that more of the smaller molecule, A, was synthesised than the larger A'. If a single mRNA served as a template for both proteins of gene A, a simple explanation for this observation could be that A' is translated only from the new mRNA while A is synthesised from old mRNA. This model would predict that synthesis of A' but not A would be prevented by preventing phage mRNA synthesis.

Preliminary experiments with cells irradiated with ultraviolet light before infection (to eliminate host protein synthesis^{11,12}) indicated that A' synthesis was selectively prevented when rifampicin of 200 μ g ml⁻¹ was added various times after infection (1, 5, 10 and 15 min). To verify that this effect was associated with gene A function, DNA replication experiments were planned. Therefore, the rifampicin effect was then examined in mitomycin C-pre-

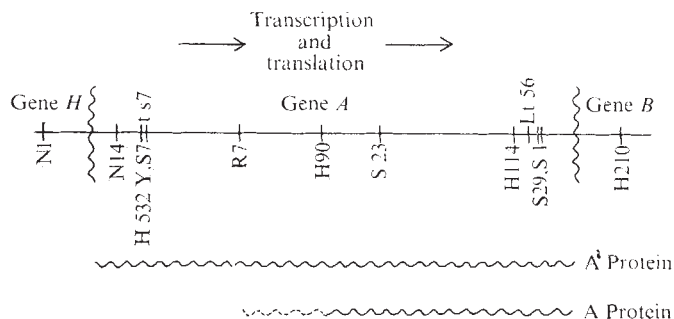


Fig. 1 A model representing synthesis of two proteins from gene A of Φ X174. The presumed internal initiator would be between the R7 and H90 positions. Amber A mutants N14, S7, H532y and R7 synthesise a stable A molecule while amber A mutants H90, S23, H114, S29, and S1 do not synthesise stable A or A' molecules. L1 56 and t's-7 are temperature-sensitive gene A mutants. The transcriptional and translational directions were derived from previous studies²⁵⁻²⁸. The map of gene A was constructed by Marie N. Hayashi.

treated cells (to eliminate host DNA synthesis³) using two hosts: *E. coli* HF4704 Rif^r, a mutant resistant to rifampicin in RNA polymerase, and *E. coli* HF4704, a rifampicin-sensitive host. Figure 2a shows the effect of adding 200 μ g ml⁻¹ of rifampicin on ³H-uridine incorporation with mitomycin C-pretreated, Φ X174-infected HF4704 15 min after infection. Figure 2b illustrates the same with the rifampicin-resistant host, HF4704 Rif^r. Rifampicin selectively inhibits RNA synthesis in HF4704 cells but not in HF4704 Rif^r cells.

The same protocol was used for labelling Φ X174-specific proteins. Rifampicin (200 μ g ml⁻¹) was added 15 min after infection and ³H-lysine was added 1 min later. The infection was allowed to proceed for 15 min and then the protein was isolated and run on sodium dodecyl sulphate (SDS) polyacrylamide gels¹¹. Control cultures without rifampicin were labelled with ¹⁴C-lysine from 16 to 30 min after infection (with no mitomycin C pretreatment of cells, phage protein is primarily synthesised 15 min after infection (E. Linney, unpublished observation)). Figure 3a shows the gel patterns of protein from the infected rifampicin-resistant host. The patterns with or without rifampicin are identical. Figure 3b illustrates that with the rifampicin sensitive host A' is clearly not synthesised when rifampicin is added. All the other phage proteins, previously identified by this laboratory^{9,11}, are synthesised.

If A is translated from old mRNA, the ratio of A/A' synthesised might be expected to increase as the infection proceeds. Table 1 summarises results which illustrate this point. *E. coli* HF4704 was preirradiated with ultraviolet light to eliminate host protein synthesis. Cells were then infected with an amber mutant, N11, in the lysis gene *E*. Protein was labelled with ³H-lysine from 0-10, 10-20 and 30-40 min after infection. The protein was isolated and separated on SDS polyacrylamide gels^{9,11}. The ratio of A/A' was calculated from their regions in the gels. In addition, a 0-10 min labelled culture was washed and chased 10-30 min after infection with cold lysine. Similarly, a 10-20 min culture was washed and chased 20-40 min after infection with cold lysine. The results in Table 1 support the view that more A is synthesised than A' as the infection proceeds. Additionally the chase data support the view that A' does not chase into the smaller protein A.

Because of the many conflicting reports on the molecular weight of gene A products^{9,11,13-15}, this effect of rifampicin on the synthesis of A' was examined to see whether it correlated with gene A function. To examine this, mitomycin C-pretreated HF4704 cells were infected in the pre-

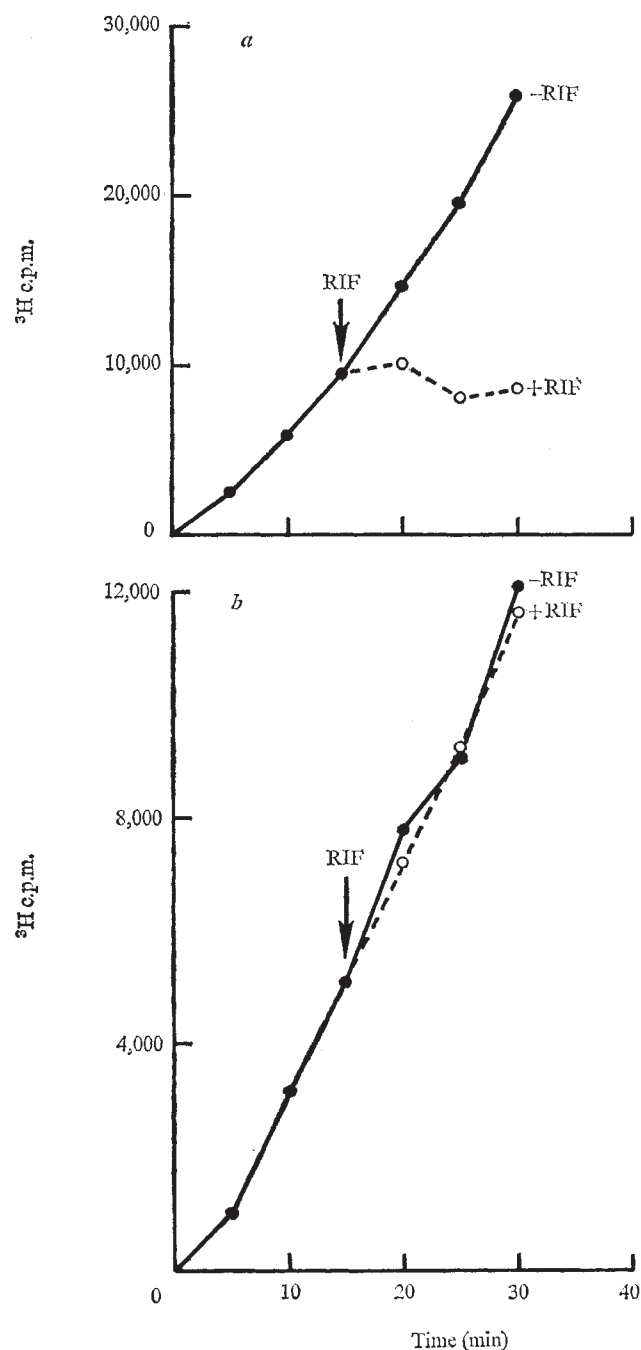


Fig. 2 Incorporation of ^3H -uridine into mitomycin C-pretreated, ΦX174 -infected cells. *E. coli* HF4704 Rif^r is a spontaneous mutant of *E. coli* HF4704 selected on rifampicin plates by Marie N. Hayashi. It was found to be rifampicin-resistant in RNA polymerase. HFC and HFB medium have been described previously¹¹. Cells were pretreated with mitomycin C at 37°C at a cell concentration of $5 \times 10^8 \text{ ml}^{-1}$ in HFC for 30 min with no agitation². Cells were then sedimented and washed once in cold HFB. Phages were adsorbed for 5 min in HFB at a cell concentration of $5 \times 10^9 \text{ ml}^{-1}$ at 37°C before starting the infection by diluting ten-fold with HFC. RNA was labelled by adding $20 \mu\text{Ci ml}^{-1}$ ^3H -uridine (New England Nuclear Corp., 26 Ci mmol^{-1}) to cultures containing $2 \mu\text{g ml}^{-1}$ uridine. Fractions ($200 \mu\text{l}$) were taken for incorporation experiments, precipitated with trichloroacetic acid (TCA) and collected on Whatman GF/C filters for scintillation counting. *a*, Host cell, HF4704: no rifampicin (●—●); $200 \mu\text{g ml}^{-1}$ rifampicin added 15 min after infection (○—○). *b*, Host cell rifampicin-resistant, HF4704 Rif^r : no rifampicin (●—●); $200 \mu\text{g ml}^{-1}$ rifampicin added 15 min after infection (○—○).

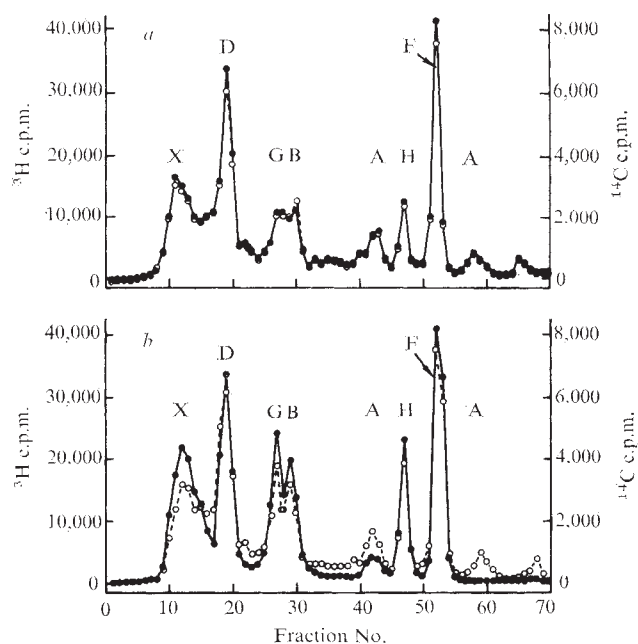


Fig. 3 SDS polyacrylamide gel of ^3H and ^{14}C -lysine of ΦX174 total protein from mitomycin C-pretreated cells labelled 16–30 min after infection. Decreasing molecular weight is to the left. The letters represent the gene products previously identified in this laboratory^{9,11}. The electrophoresis methods have been described previously⁹. For labelling protein $20 \mu\text{g ml}^{-1}$ of ^3H -lysine (Amersham-Searle, 5.36 mmol^{-1}) was added at the given time or ^{14}C -lysine (Amersham-Searle, $342 \text{ MCi mmol}^{-1}$) at an equivalent lysine concentration. *a*, Protein from ΦX174 -infected HF4704 Rif^r : $200 \mu\text{g ml}^{-1}$ rifampicin, 15 min after infection, ^3H -lysine, 16–30 min after infection (●—●); no rifampicin, ^{14}C -lysine 16–30 min after infection (○—○). *b*, Protein from ΦX174 -infected HF4704: $200 \mu\text{g ml}^{-1}$ rifampicin 15 min after infection (—); ^3H -lysine, 16–30 min after infection (●—●); no rifampicin, ^{14}C -lysine, 16–30 min after infection (○—○).

sence of $200 \mu\text{g ml}^{-1}$ chloramphenicol to allow parental RF synthesis^{1,10} and mRNA synthesis (unpublished observations of M.H.) but to inhibit protein synthesis. After 10 min of infection the chloramphenicol was washed out in the presence and in the absence of $200 \mu\text{g ml}^{-1}$ of rifampicin. After washing, the infection was allowed to proceed in the presence or absence of $200 \mu\text{g ml}^{-1}$ of rifampicin, respectively. Thus, the rifampicin culture would have to rely on old mRNA for gene A function. Figure 4 illustrates ^3H -thymidine incorporation under these conditions. In the presence of chloramphenicol for 30 min, DNA replication was inhibited while if the chloramphenicol was removed in the absence of rifampicin DNA replication proceeded. If chloramphenicol was replaced with rifampicin, DNA replication remains inhibited.

Thus, phage DNA replication is prevented when it is made dependent on old phage mRNA. That this effect is related to the absence of A' synthesis and not the absence of a necessary newly synthesised host protein is suggested by previous findings that in cells preirradiated with ultraviolet light to eliminate host protein synthesis, phage DNA replication occurs¹¹.

The results indicate that A' is necessary for gene A function. The tight coupling between translation and transcription could be responsible for the *cis* effect of gene A. Since both A and A' have been shown to bind tightly to DNA cellulose columns⁹, if A' is synthesised at the DNA template as the tight coupling suggests, this affinity might increase the possibility of A' remaining there and not diffusing to another phage DNA molecule.

Because the smaller gene *A* protein, A, and the other identified phage proteins are synthesised in the presence of rifampicin the results suggest that the 5' end of the gene *A* message is unique. A promoter is presumed to be located at the beginning of gene *A*^{17,18} because the observed gene *F* to gene *H* polarity has not been found to extend to gene *A*. Although Φ X174 mRNA has been found to be structurally stable in a macromolecular sense¹⁷, it has been shown¹⁹ that the functional half life of gene *F* mRNA from the related phage S13 follows a two-component curve: about 70% of functional message decays with a half life ($t_{1/2}$) of approximately 1.4 min and 30% with a $t_{1/2}$ of approximately 14 min. Since a promoter site is presumed to be at the beginning of gene *F* (ref. 18), the $t_{1/2}$ of approximately 1.4 min was suggested to be caused by rapid degradation of the 5' end of the gene *F* message¹⁹. This model of degradation from the promoter end of phage mRNA¹⁹ is consistent with our results for A' synthesis. Quantitation of A' synthesis immediately after the addition of rifampicin is impossible at this point in time because it

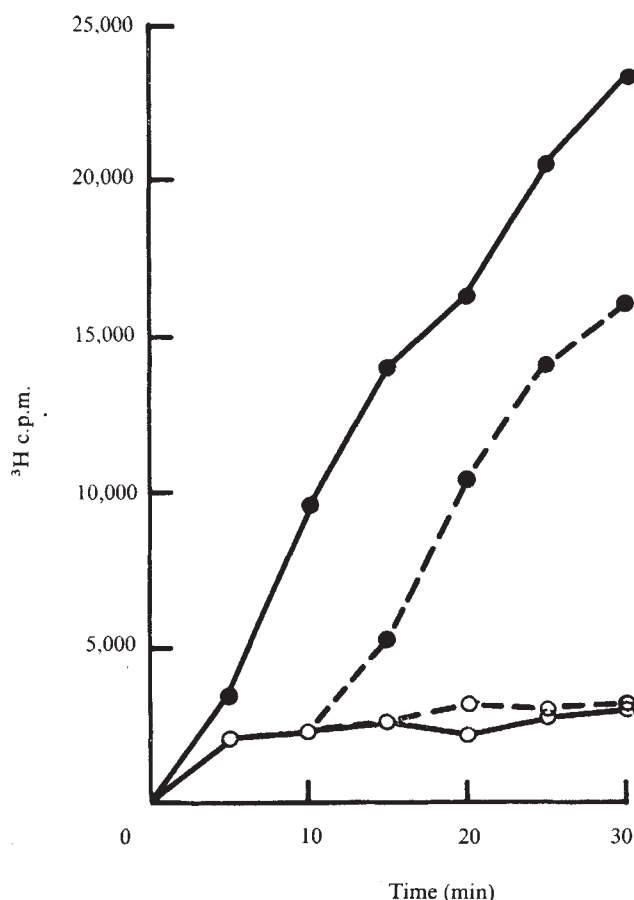


Fig. 4 ^3H -thymidine incorporation into mitomycin C-pretreated HF4704, Φ X174-infected cultures. Chloramphenicol (a gift from Parke-Davis Co.) was used at a concentration of $200\ \mu\text{g ml}^{-1}$. Cells were washed twice with HFC with ^3H -thymidine plus or minus rifampicin to remove the chloramphenicol. Rifampicin (Calbiochem) dissolved in dimethyl sulphoxide ($20\ \text{mg ml}^{-1}$) was used at a concentration of $200\ \mu\text{g ml}^{-1}$. DNA was labelled by adding $20\ \mu\text{Ci ml}^{-1}$ of ^3H -thymidine (New England Nuclear Corp., $6.7\ \text{Ci mmol}^{-1}$) was added to the media containing $5\ \mu\text{g ml}^{-1}$ thymine. No chloramphenicol (●—●); $200\ \mu\text{g ml}^{-1}$ chloramphenicol from 0 to 30 min after infection (O—O); chloramphenicol present from 0 to 10 min after infection and then washed out (●---●); chloramphenicol washed out in the presence of $200\ \mu\text{g ml}^{-1}$ of rifampicin 10 min after infection and the infection then allowed to proceed in the presence of rifampicin (O---O).

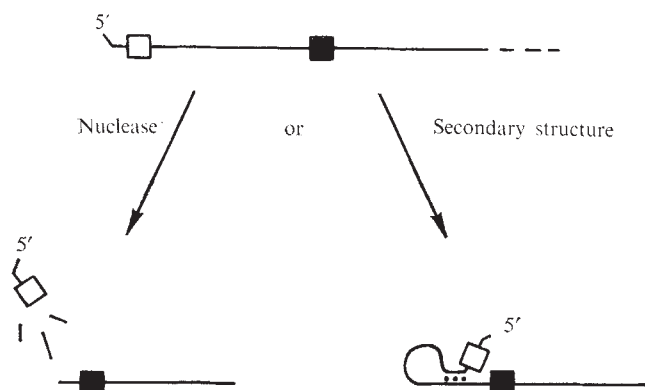


Fig. 5 Two models for gene *A* on RNA. □ A' translational initiation site; A translational initiation site.

is a minor peak in the total gel profile. When rifampicin and ^3H -lysine are added together, 15 min after infection, no detectable A' is synthesised. The possibility remains that a small base-pairing region at the 5' end of gene *A* message rather than selective degradation at the end could explain this phenomenon. However, the decrease in A synthesis after the addition of rifampicin (see Fig. 3b) supports the degradation model (Fig. 5). It is still not known whether there exists a promoter internal to gene *A* that might also explain the synthesis of the smaller gene *A* protein. If this were the correct explanation there would still be different mRNA stabilities for the A' and A mRNAs.

Table 1 Ratio of ^3H -lysine c.p.m. in A and A' from *E. coli* HF4704 preirradiated with ultraviolet light to eliminate host protein synthesis^{11,12} and then infected with the Φ X174 lysis mutant, N11

Conditions	A c.p.m./A' c.p.m.
0-10 min pulse	1.9
10-30 min chase	1.9
10-20 min pulse	2.5
20-40 min chase	2.6
30-40 min pulse	2.8

The effect of rifampicin on Φ X174 DNA replication has been described before. Although it does not prevent complementary strand synthesis on the incoming single-stranded DNA molecule²⁰, as has been found for M13 (ref. 21), it has been shown to inhibit phage DNA replication when added before infection²². Addition of rifampicin later in infection allowed replication to occur. The assumption made in ref. 22 was that gene *A* mRNA was long lived and sufficient mRNA was available for gene *A* to function after the addition of rifampicin. The results are consistent with ours but our interpretation would be that once gene *A* is allowed to function, new A' is not necessary for continued phage DNA replication.

Although the results suggest that the larger of the two gene *A* proteins is necessary for gene *A* function, they provide no explanation for the smaller protein, A. For reasons we cannot explain this molecule is not clearly separated from gene *H* protein when *bis* acrylamide is used as a cross-linker in SDS polyacrylamide gels. Since *bis* acrylamide has been commonly used by the other laboratories, our use of ethylene diacrylate as a cross-linker which clearly separates A and H (see Fig. 3a and b) explains why no other laboratory has associated a 35,000 molecular weight protein with gene *A*. No function has been established for A, though a minor virion component (approximately one molecule per particle¹⁹) described previously^{13,23,24} has been

found to coelectrophorese with A isolated from single-stranded DNA cellulose columns.

This work was supported, in part, by grants from the US Public Health Service and the US National Science Foundation. M. H. is the recipient of a research career development award from the US Public Health Service.

ELWOOD LINNEY*
MASAKI HAYASHI

Department of Biology,
University of California, San Diego,
La Jolla, California 92037

*Present address: Department of Microbiology, State University of New York, Stony Brook, New York 11790.

Received December 3, 1973.

- ¹ Tessman, E. S., *J. molec. Biol.*, **17**, 218 (1966).
- ² Lindqvist, B. H., and Sinsheimer, R. L., *J. molec. Biol.*, **30**, 69 (1967).
- ³ Iwaya, M., and Denhardt, D. T., *J. molec. Biol.*, **57**, 159 (1971).
- ⁴ Denhardt, D. T., Dressler, D. H., and Hathaway, A., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 803 (1967).
- ⁵ Tessman, E. S., *Virology*, **25**, 303 (1965).
- ⁶ Francke, B., and Ray, D. S., *J. molec. Biol.*, **61**, 565 (1971).
- ⁷ Francke, B., and Ray, D. S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 475 (1972).
- ⁸ Baas, D. D., and Jansz, H. S., *J. molec. Biol.*, **63**, 569 (1972).
- ⁹ Linney, E. A., Hayashi, M. N., and Hayashi, M., *Virology*, **50**, 381 (1972).
- ¹⁰ Linney, E., and Hayashi, M., *Nature new Biol.*, **245**, 6 (1973).
- ¹¹ Gelfand, D. H., and Hayashi, M., *J. molec. Biol.*, **44**, 501 (1969).
- ¹² Burgess, A. B., and Denhardt, D. T., *J. molec. Biol.*, **44**, 377 (1969).
- ¹³ Godson, G. N., *J. molec. Biol.*, **57**, 541 (1971).
- ¹⁴ Benbow, R. M., Mayol, R. F., Picchi, J. C., and Sinsheimer, R. L., *J. Virol.*, **10**, 99 (1972).
- ¹⁵ Van der Mei, D., Zandberg, J., and Jansz, H. S., *Biochim. biophys. Acta*, **287**, 312 (1972).
- ¹⁶ Sinsheimer, R. L., Hutchison, C. A., and Lindqvist, B., in *Molecular biology of viruses*, 175 (Academic Press, New York 1967).
- ¹⁷ Hayashi, Y., and Hayashi, M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 171 (1970).
- ¹⁸ Vanderbilt, A. S., Borrás, M. T., Germeraad, S., Tessman, I., and Tessman, E. S., *Virology*, **50**, 171 (1972).
- ¹⁹ Puga, A., Borrás, M. T., Tessman, E. S., and Tessman, I., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2171 (1973).
- ²⁰ Silverstein, S., and Billew, D., *Biochim. biophys. Acta*, **247**, 383 (1971).
- ²¹ Brutlag, D., Schekman, R. W., and Kornberg, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2826 (1971).
- ²² Van der Mei, D., Brons, J. Th., and Jaisz, H. S., *Biochim. biophys. Acta*, **262**, 463 (1972).
- ²³ Suruda, A. J., and Poljak, R. J., *Virology*, **46**, 164 (1971).
- ²⁴ Zuccarelli, A., Benbow, R. M., and Sinsheimer, R. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1905 (1972).
- ²⁵ Hayashi, M., Hayashi, M. N., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 664 (1963).
- ²⁶ Vanderbilt, A. S., and Tessman, I., *Nature*, **228**, 54 (1971).
- ²⁷ Tessman, I., Kumar, S., and Tessman, E. S., *Science*, **158**, 267 (1967).
- ²⁸ Vanderbilt, A. S., Borrás, M., and Tessman, E. S., *Virology*, **43**, 352 (1971).

Requirement for either DNA polymerase I or DNA polymerase III in post-replication repair in excision-proficient *Escherichia coli*

WHEN *Escherichia coli* bacteria deficient in excision repair (*uvr*) replicate their DNA after ultraviolet irradiation the newly-synthesised DNA contains gaps which are rejoined during subsequent incubation¹ by a postreplication repair process believed to be recombinational in nature². Double mutants (*uvr recA*) deficient in both excision and post-replication repair are up to 25 times more sensitive to ultraviolet than those deficient in excision alone³. Bacteria deficient in excision repair and DNA polymerase I (*uvr polA*) in contrast are no more

ultraviolet-sensitive than those deficient solely in excision⁴, which might suggest that DNA polymerase I is not required for successful post-replication repair. The fact that DNA polymerase III seems to be able to substitute for DNA polymerase I in the repair of excision gaps⁵, and type II DNA strand breaks⁶ after X irradiation, however, indicates that DNA polymerase I cannot be excluded from involvement in post-replication repair if similar substitution by DNA polymerase III could occur there.

We have therefore examined the filling and joining of daughter-strand gaps in DNA synthesised after exposure to ultraviolet of bacteria deficient in DNA polymerase I (*polA*) and possessing temperature-sensitive DNA polymerase III activity (*dnaE*). The results are consistent with a requirement for either DNA polymerase I or DNA polymerase III in the gap-filling process.

We used *E. coli* BT1026 *dnaE polA-1 thy* (ref. 7) and 1026-1 which is a *polA*⁺ MMS-resistant revertant isolated by W. Goebel. Some experiments were also carried out with a related strain BT1126 *dnaE polA-1 thy* (ref. 8) which carries a different *dnaE* mutation. All these strains are deficient in endonuclease I. The bacteria were grown at 33° C (permissive temperature) to about 2×10^8 ml⁻¹ in M9 minimal medium supplemented with 1% casein hydrolysate and 2 µg ml⁻¹ thymine and exposed to ultraviolet. They were then pulse labelled at 33° C by the addition of 100 µCi (³H-methyl) thymidine ml⁻¹ for a period of 7.5 to 12.5 min, filtered on membrane filters, washed with ten volumes of prewarmed buffer, and resuspended in unlabelled medium. The length of the pulse was adjusted to ensure that label uptake was not too dissimilar in irradiated and un-irradiated cultures to minimise end-error artefact⁹. The molecular weight of the DNA synthesised during the pulse was examined by centrifugation through an alkaline sucrose density gradient immediately after the pulse and at various times during subsequent incubation of the bacteria at 33° C or 44° C (restrictive temperature for *dnaE*).

Strain 1026-1 is phenotypically *PolA*⁺ *DnaE*⁺ at 33° C and, as expected, filled gaps in daughter-strand DNA efficiently within 20 min at that temperature (Fig. 1b, c). At 44° C, daughter-strand gaps were also joined within 20 min. (Fig. 1d) indicating that postreplication repair can occur with no noticeable loss of efficiency in the absence of DNA polymerase III activity. It also shows that continued normal semiconservative replication is not necessary for post-replication gap filling.

As found with other *polA* bacteria¹⁰, DNA synthesised in BT1026 at 33° C did not reach normal size as fast as in *polA*⁺

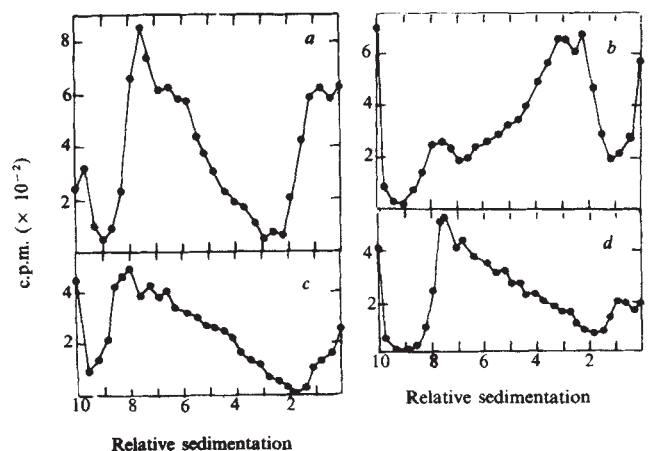


Fig. 1 Alkaline sucrose gradient sedimentation profiles of newly-synthesised DNA from *E. coli* 1026-1. a No ultraviolet, 7.5 min ³H-thymidine pulse; b 100 ergs mm⁻² ultraviolet, 10 min ³H-thymidine pulse; c, 100 ergs mm⁻² ultraviolet, 10 min ³H-thymidine pulse, 20 min incubation at 33° C; d, 100 ergs mm⁻² ultraviolet, 10 min ³H-thymidine pulse, 20 min incubation at 44° C.

bacteria and about 20 min of subsequent incubation were needed before the normal profile was obtained (Fig. 2a). Further incubation did not result in any further increase in size. DNA synthesised in irradiated BT1026 was of smaller size. After 80 min incubation at 33° C, however, the DNA had been joined together to form pieces of normal size (Fig. 2c). Thus at 33° C, where BT1026 is phenotypically *PolA*⁻ *DnaE*⁺, daughter-strand gaps could be filled indicating that a deficiency of DNA polymerase I does not prevent postreplication gap-filling although the process is slower in this strain.

In BT1126 there was postreplication repair during 30 min incubation at 33° C (Fig. 3c) indicating that in this strain the polymerase I deficiency did not markedly slow down the rate of gap filling. Other experiments have shown that *polA dnaE*⁺ bacteria are able to carry out gap filling at 44° C (data not shown).

In contrast, in both BT1026 and BT1126 at 44° C, where the bacteria were phenotypically *PolA*⁻ *DnaE*⁻, DNA that had been synthesised on an irradiated template (Fig. 3b) never increased to normal size and was degraded (Figs 2d and 3d). Degradation was faster in BT1126, the smaller pieces being completely acid-soluble within 15 min. Thus where both polymerase I and polymerase III were inactive there was no detectable filling of daughter-strand gaps. If there was any residual repair (perhaps involving DNA polymerase II) it was very limited in extent and did not give rise to DNA of normal size.

An interesting observation with BT1026 was that when unirradiated bacteria were transferred to 44° C after being pulse labelled at 33° C, the initially small DNA increased in

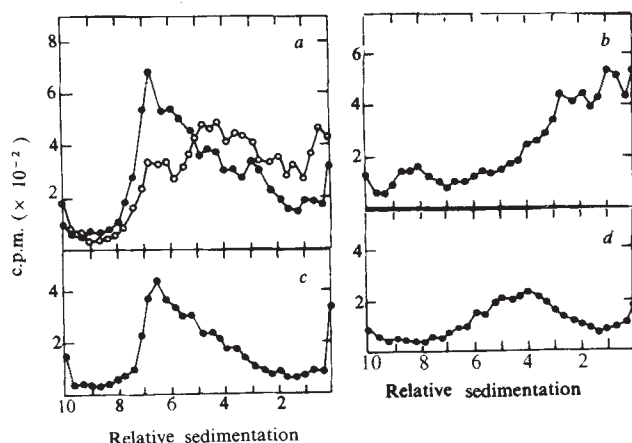


Fig. 2 Alkaline sucrose gradient sedimentation profiles of newly-synthesised DNA from *E. coli* 1026. *a*, No ultraviolet, 7.5 min ³H-thymidine pulse with no (●) or 20 min (○) incubation at 33°; *b*, no ultraviolet, 7.5 min ³H-thymidine pulse, with 20 min (●) or 80 min (○) incubation at 44°; *c*, 100 ergs mm⁻² ultraviolet, 12.5 min ³H-thymidine pulse, no (●), 20 min (○) or 80 min (▲) incubation at 33°; *d*, 100 ergs mm⁻² ultraviolet, 12.5 min ³H-thymidine pulse, 20 min (●) or 80 min (○) incubation at 44° C.

size and sedimented at essentially the normal molecular weight after 20 min incubation but further incubation to 80 min resulted in a decrease in size without any loss of counts (Fig. 2b). We have no explanation for this observation.

We conclude that ultraviolet-irradiated bacteria deficient in either DNA polymerase I or DNA polymerase III are able to carry out postreplication gap filling. Bacteria deficient in both polymerases show little or no postreplication gap filling and tend to degrade newly-synthesised DNA. The results are consistent with the hypothesis that the two polymerases can substitute one for the other in daughter-strand gap filling, as in repair replication after excision repair⁵. The data do not give any indication as to which is the normal polymerase in conditions in which both enzymes are present and potentially active.

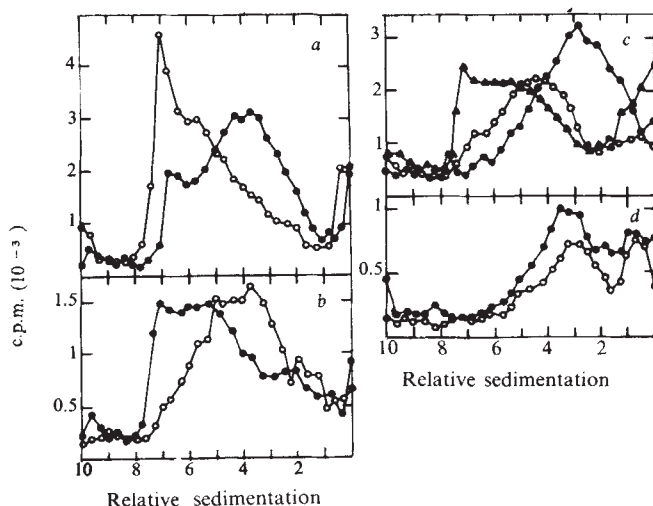


Fig. 3 Alkaline sucrose gradient sedimentation profiles of newly-synthesised DNA from *E. coli* BT1126. *a*, No ultraviolet, 7.5 min ³H-thymidine pulse with no (●) or 30 min (○) incubation at 33° C; *b*, 100 ergs mm⁻² ultraviolet, 10 min ³H-thymidine pulse; *c*, 100 ergs mm⁻² ultraviolet, 10 min ³H-thymidine pulse, 30 min incubation at 33° C; *d*, 100 ergs mm⁻² ultraviolet, 10 min ³H-thymidine pulse, 45 min incubation at 44° C.

It should be emphasised that these results have been obtained with excision-proficient strains. Their postreplication repair process may conceivably be different from that in excision-deficient strains. It is possible, for example, to formulate a model for excision-proficient bacteria in which photoproducts are excised from recently replicated parental DNA using the homologous parental strand transiently rehybridised with it as the template for repair replication. Such a process might be expected to require both excision and recombination genes. Circumstantial evidence for a process with such requirements has been recently reported¹¹.

S. G. Sedgwick acknowledges receipt of a MRC Scholarship. We thank David Sherratt for strains and Michael Green for helpful discussion.

S. G. SEDGWICK*
B. A. BRIDGES

*School of Biological Sciences and
MRC Cell Mutation Unit,
University of Sussex,
Falmer, Brighton, BN1 9QG*

*Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Received December 10, 1973; revised February 6, 1974.

- Rupp, W. D., and Howard-Flanders, P., *J. molec. Biol.*, **31**, 291 (1968).
- Rupp, W. D., Wilde, E. E., Reno, D. L., and Howard-Flanders, P., *J. molec. Biol.*, **61**, 25 (1971).
- Howard-Flanders, P., and Boyce, R. P., *Radiation Res.*, Supplement, **6**, 156 (1966).
- Monk, M., Peacy, M., and Gross, J. D., *J. molec. Biol.*, **58**, 623 (1971).
- Youngs, D. A., and Smith, K. C., *Nature new Biol.*, **244**, 241 (1973).
- Youngs, D. A., and Smith, K. C., *J. Bact.*, **114**, 851 (1973).
- Geffer, M., Hirst, Y., Kornberg, T., Barnoux, C., and Weschler, J. A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 3150 (1971).
- Weschler, J. A., Nusslein, V., Otto, B., Klein, A., Bonhoeffer, F., Herrman, R., Gloger, L., and Schaller, H., *J. Bact.*, **113**, 1381 (1973).
- Lehmann, A. R., and Ormerod, M. G., *Nature*, **221**, 1053 (1969).
- Okazaki, R., Arisawa, M., and Sugino, A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2754 (1971).
- Bridges, B. A., *Nature new Biol.*, **240**, 52 (1972).

Is the cytoplasm a site of nuclear DNA synthesis?

OBSERVATIONS which do not harmonise with the original self-replicative scheme of DNA synthesis have received little attention. In particular, interest in cytoplasmic DNA synthesis in eukaryotic cells has been limited to semiautonomous structures which contain DNA templates. But it was pointed out¹ as early as 1958 that transfer of genetic information between DNA, RNA and protein often can proceed in either direction. It is possible that an initial step of DNA synthesis, corresponding for example to RNA-primed synthesis of Okazaki fragments^{2,3}, may generally occur out of cell nuclei and without direct participation of a DNA template.

cytoplasmic fraction by nuclear DNA during the homogenate fractionation. I have therefore come to the conclusion that the cytoplasmic DNA synthesis may represent a prenuclear stage of the genome replication.

I thank E. Kraszewska for technical assistance. This work was supported by the Polish Academy of Sciences and, in part, by the US Department of Agriculture, Agricultural Research Service.

J. BUCHOWICZ

*Polska Akademia Nauk,
Instytut Biochemii i Biofizyki,
02-532 Warszawa, Poland*

Received November 28, 1973; revised February 20, 1974.

¹ Chargaff, E., in *The Origin of Life on the Earth* (edit. by Oparin, A. I.), 297 (Pergamon Press, London, 1959).

Table 1 Incorporation of ¹⁴C-thymidine into nuclear, organellar and cytoplasmic DNA fractions of germinating wheat embryos

Germination time (h)	DNA amount and radioactivity (per 100 embryos)					
	(μg)	Nuclear (d.p.m.)	(μg)	Organellar (d.p.m.)	(μg)	Cytoplasmic (d.p.m.)
3	125	< 10	15	< 10	< 5	< 10
6	120	250	20	< 10	< 5	220
12	140	640	25	40	< 5	210
24	170	1,350	20	150	< 5	120

A sample of 350 wheat grains was surface sterilised with 2% NaClO and left at 2° C for 8 h with 10 ml of ¹⁴C-thymidine solution (1.0 μCi ml⁻¹, 53 μCi-μmol⁻¹). The prelabelled sample was washed with water and allowed to germinate at 22° C for the indicated time. Embryos were then separated and ground in a mortar with 10 ml of ice-cold 0.25 M sucrose containing 15 mM potassium phosphate (pH 7.3) and 10 mM MgCl₂. The homogenate was filtered through a nylon tissue and fractionated by centrifugation. Nuclear (1,000g pellet), organellar (18,000g pellet) and cytoplasmic (18,000g supernatant) fractions were obtained and washed with 0.3 M HClO₄ containing 1 mM thymidine. RNA was removed by an alkaline hydrolysis (0.3 M KOH, 37° C, 15 h) and DNA fraction obtained as an acid hydrolysate (0.5 M HCl, 100° C, 30 min). The acid hydrolysate was applied to paper chromatography in a water-saturated butan-1-ol solvent system¹⁰. Adenine and guanine were eluted from the corresponding spots and determined spectrophotometrically to calculate the DNA content. The origin, containing a mixture of pyrimidine nucleotides resulting from the acid hydrolysis of DNA, was used for the measurement of radioactivity in a Packard liquid scintillation counter.

We tested for a prenuclear stage of DNA formation in germinating wheat embryo, in which biosynthetic events triggered after the breaking of dormancy are naturally synchronised⁴. It has been observed⁵ that incorporation of thymidine into DNA of germinating wheat seeds begins only after some preliminary activation of RNA synthesis is completed, implying an RNA-dependent DNA synthesis in this system. To check whether the cytoplasm may contribute to the early stage of DNA synthesis, wheat grains were prelabelled with ¹⁴C-thymidine and germinated for various periods. Total amounts and radioactivities of nuclear, organellar and cytoplasmic DNA of the embryo were then determined (Table 1).

I found that after 6 h germination radioactive DNA appeared in the nuclear and soluble cytoplasmic fractions (Table 1). Specific radioactivity of the cytoplasmic DNA was, however, at least 20-fold higher than that of the nuclear DNA. As germination proceeded further the radioactivity of the cytoplasmic DNA decreased considerably and that of the nuclear DNA rose correspondingly. These observations are consistent with the hypothesis of a precursor-product relationship between the cytoplasmic and nuclear DNA. Rapid activation of cytoplasmic DNA synthesis in an early response to proliferative stimuli has been observed in other eukaryotic cells⁶⁻⁸. The experimental data do not exclude the possibility of an independent synthesis of the cytoplasmic and nuclear DNA. But the only explicable reason for the synthesis of deoxyribopolynucleotides in the supernatant fraction of the cytoplasm, which is virtually free of mature DNA molecules, is to supply them to the nucleus and other DNA-containing structures.

Another explanation is the transport of informational DNA from nucleus to cytoplasm, which has been reported for cultured animal cells⁹. This explanation, however, can be hardly extended to the germinating wheat embryo, where radioactivity of the cytoplasmic DNA decreases after the nuclear DNA becomes highly radioactive. The same observation seems to exclude the possibility of a significant contamination of the

² Okazaki, R., Okazaki, T., Sakebe, K., Sugimoto, K., Sugino, A., and Iwatsuki, N., *Cold Spring Harbor Symp. quant. Biol.*, **33**, 129 (1968).

³ Okazaki, R., Sugino, A., Hirose, S., and Tamanoi, F., *Ninth Int. Congress of Biochem.*, Stockholm, 126 (1973).

⁴ Dobrzanska, M., Tomaszewski, M., Grzelczak, Z., Rejman, E., and Buchowicz, J., *Nature*, **244**, 507 (1973).

⁵ Rejman, E., and Buchowicz, J., *Phytochemistry*, **10**, 2951 (1971).

⁶ Hanson, K. P., Ivanova, L. V., Nikitina, Z. S., Shutko, A. N., and Komar, V. E., *Biokhimiya*, **35**, 635 (1970).

⁷ Chang, L. M. S., and Bollum, F. J., *J. biol. Chem.*, **247**, 7958 (1972).

⁸ Chang, L. M. S., Brown, M. K., and Bollum, F. J., *J. molec. Biol.*, **74**, 1 (1973).

⁹ Koch, J., and Pfeil, H. V., *FEBS Lett.*, **24**, 53 (1972).

¹⁰ Buchanan, J. G., *Nature*, **168**, 1091 (1951).

Effect of 5-bromodeoxyuridine on the size distribution of DNAs isolated from sea urchin embryos

5-BROMODEOXYURIDINE (BUdR), an analogue of thymidine, has been shown to selectively inhibit cell differentiation in various cell types and development of embryos, without affecting cell maintenance capabilities¹. Gontcharoff and Mazia² demonstrated that exposure of newly fertilised sea urchin eggs or early cleavage stage embryos to BUdR resulted in embryos which did not gastrulate or produce echinochrome pigment. From this laboratory evidence has been reported³ for a BUdR-induced modification of DNA replication in developing sea urchin embryos. Pulse-chase experiments in sea urchin gastrulae demonstrated that newly synthesised low molecular weight (8-15S) nuclear DNA labelled with ³H-BUdR was not readily chased by unlabelled BUdR into higher molecular weight DNA. In contrast, 8-15S DNA labelled with ³H-thymidine was converted into higher molecular weight DNA during a relatively short chase period with unlabelled thymidine. This data suggested the incorporation of BUdR into newly synthesised low

molecular weight DNA molecules in embryos might prevent or slow the rate of normal linkage of such molecules into higher molecular weight DNA.

We report here that morula and blastula stage sea urchin embryos grown continuously from fertilisation in the presence of BUdR accumulate DNA molecules (30-60S) which are not integrated into higher molecular weight bulk DNA.

The effect of BUdR on the size distribution of isolated long term ^{32}P -labelled DNA from embryos was determined on neutral sucrose gradients. A control culture of morula stage embryos grown continuously from fertilisation in the presence of ^3H -thymidine was collected and mixed with a culture of morula stage embryos similarly labelled with ^{32}P but grown continuously in the presence of either $50\text{ }\mu\text{g ml}^{-1}$ BUdR or $50\text{ }\mu\text{g ml}^{-1}$ thymidine (TdR). DNA was coisolated from mixtures of ^3H - and ^{32}P -labelled embryos and centrifuged through a neutral sucrose gradient as described in the legend to Fig. 1. ^{32}P -labelled morula stage DNA isolated from embryos grown in the presence of $50\text{ }\mu\text{g ml}^{-1}$ TdR sedimented with a distribution similar to ^3H -labelled morula stage DNA coisolated from embryos grown without added nucleoside (Fig. 1a). In contrast, a portion of ^{32}P -labelled morula stage DNA isolated from embryos grown in the presence of $50\text{ }\mu\text{g ml}^{-1}$ BUdR sedimented more slowly than ^3H -labelled morula DNA coisolated from the control culture (Fig. 1b). Similar differences in sedimentation distribution of control DNAs and DNAs isolated from embryos grown in the presence of BUdR were obtained from blastula stage embryos.

A summary of data demonstrating the effect of BUdR on the size distribution of ^{32}P -labelled morula and blastula stage DNAs on neutral sucrose gradients is presented in Table 1. In addition to the coisolation and sedimentation of BUdR and control DNAs as shown in Fig. 1, long term ^{32}P -labelled DNAs were isolated separately and consedimented with marker ^3H -DNA from bacteriophage $\Phi 80$. This facilitated a determination of the proportion of different size ranges of sea urchin DNA compared with the position of the marker DNA. A portion of the ^{32}P -labelled DNAs isolated from both morula and blastula stage embryos grown in the presence of BUdR sediments more slowly than ^{32}P -labelled DNA isolated from control cultures. The difference is represented by a decrease in the proportion of DNA sedimenting at greater than 60S and a corresponding increase of 8-10% in the morula and 3-5% in the blastula stage DNA sedimenting in a range from 30-60S. That this difference in sedimentation distribution is specifically the result of the presence of BUdR during embryogenesis rather than excess deoxyribonucleosides in the culture medium is demonstrated by the close similarity between the sedimentation distribution of DNA isolated from control cultures and those in which the embryos were grown in $50\text{ }\mu\text{g ml}^{-1}$ TdR (Fig. 1a and Table 1).

To ensure that the difference in sedimentation of ^{32}P -labelled DNAs obtained from embryos grown in the presence of BUdR (compared with control DNAs) was not the result of ^{32}P -containing molecules other than DNA, samples of the sea urchin DNA preparations labelled with ^{32}P were subjected to enzymatic and alkaline treatment. More than 90% of the ^{32}P was resistant to a mixture of pancreatic and T_1 RNases; more than 95% of the radioactivity was DNase-sensitive; more than 85% of the ^{32}P was resistant to 0.2 M NaOH at 60°C for 1 h. Thus at least 85% of the ^{32}P recovered was in DNA. More importantly, ^{32}P -labelled morula stage DNA isolated from embryos grown in the presence of BUdR was incubated with or without the addition of DNase and then centrifuged through neutral sucrose gradients (data not shown). The small percentage of DNase-resistant ^{32}P was found near the top of the gradient. Therefore, we believe that the shift in distribution of ^{32}P observed in the sedimentation profiles of DNAs isolated

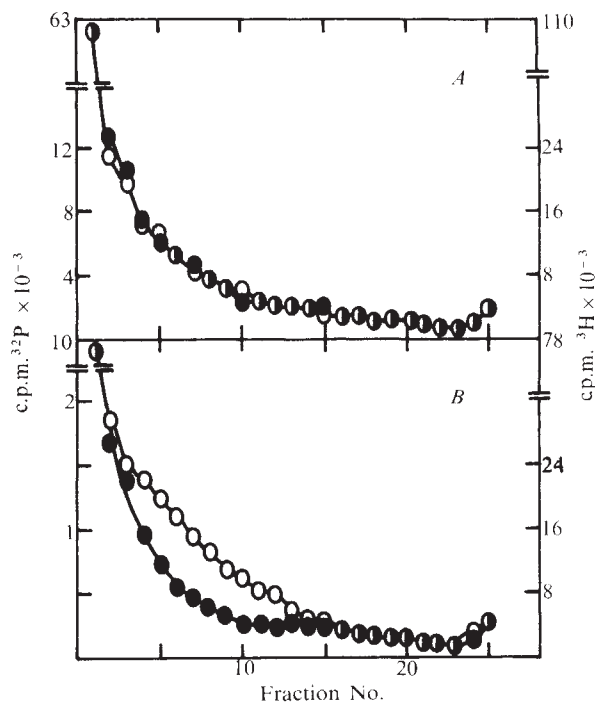


Fig. 1 Comparison on neutral sucrose gradients of profiles of control DNAs and DNAs synthesised in the presence of BUdR. *Strongylocentrotus purpuratus* embryos were cultured³ in artificial seawater¹¹ containing penicillin and streptomycin to the morula stage of development: *A*, DNA coisolated from embryos grown under normal conditions in the presence of $2\text{ }\mu\text{Ci ml}^{-1}$ ^3H -methyl-thymidine (20 Ci mmol^{-1}) and then mixed after growth with embryos grown in the presence of $50\text{ }\mu\text{g ml}^{-1}$ TdR with $1\text{ }\mu\text{Ci ml}^{-1}$ ^{32}P ; *B*, DNA coisolated from control embryos grown under normal conditions in the presence of ^3H -TdR and then mixed with embryos grown in the presence of $50\text{ }\mu\text{g ml}^{-1}$ BUdR and $1\text{ }\mu\text{Ci ml}^{-1}$ ^{32}P . In all comparative experiments cultures were derived from a single batch of eggs pooled from several females. Nucleosides were added immediately after fertilisation and radioisotopes were added 10 min after fertilisation, all being present until the embryos were collected. Each 100 ml culture of embryos was killed by the addition of an equal volume of 20% phenol (freshly redistilled, saturated with 50 mM sodium phosphate buffer, $\text{pH } 7.0$) in -20°C ethanol. The embryos were pelleted from the alcohol-phenol solution by centrifugation at $10,000\text{ g}$ for 15 min and resuspended in 100 ml of 100% ethanol. After an additional centrifugation, embryos were resuspended and combined in a total of 10 ml of buffer which was 50 mM Tris, 50 mM EDTA, $\text{pH } 8.5$ at 37°C . Cells were lysed by the addition of sodium dodecylsulphate to 0.5% . Pronase (predigested 1.5 h at 37°C in Tris-EDTA buffer) was added to 1 mg ml^{-1} and the lysate was kept overnight at 37°C . α -Amylase (Worthington, twice crystallised) was added to $100\text{ }\mu\text{g ml}^{-1}$ and pancreatic RNase (Worthington, stock solution is 2 mg ml^{-1} in 0.15 M NaCl , heated to 85°C for 20 min) to $20\text{ }\mu\text{g ml}^{-1}$; the mixture was then incubated at 37°C for 2 h. Predigested pronase was then added to $100\text{ }\mu\text{g ml}^{-1}$ and the DNA was held at 37°C overnight. The resulting DNA solution was extracted three times with phenol and extensively dialysed against $1 \times \text{SSC}$ (SSC is 0.15 M NaCl , 0.015 M sodium citrate). Approximately $45\text{ }\mu\text{g}$ of the ^3H - and ^{32}P -labelled coisolated DNA was brought to a final volume of 1.0 ml in $1 \times \text{SSC}$ and layered onto a $30\text{ ml } 5\text{-}20\%$ sucrose gradient containing 1 M NaCl , 20 mM Tris-HCl , 1 mM EDTA , $\text{pH } 7.3$. Centrifugation was in a Beckman SW 25.1 rotor for 10 h at $24,000\text{ r.p.m.}$ at 20°C . Fractions were collected from the bottom of the gradient and precipitated at 4°C by the addition of 2 volumes of 10% trichloroacetic acid and $25\text{ }\mu\text{g}$ bovine serum albumen. Fractions were collected on Whatman GF/A filters, dried, and counted in toluene-based scintillation fluid. O, ^{32}P ; ●, ^3H .

from embryos grown in the presence of BUdR (Fig. 1b and Table 1) represents ^{32}P -containing DNA.

It should be noted that the portion of isolated bulk DNA which contains radioactivity represents only nuclear DNA, for mitochondrial DNA replication does not occur in sea urchin embryos until after gastrulation^{4,5}.

Table 1 Summary of data demonstrating an effect by BUdR on sedimentation in neutral sucrose gradients of ^{32}P -labelled DNAs isolated from sea urchins embryos.

$S_{20,w}$ distribution*	Calculated† molecular weight $\times 10^{-6}$	% of total counts					
		Morula			Blastula		
		Control	BUdR	TdR	Control	BUdR	TdR
> 60	> 154	47.9	38.7	50.4	70.3	64.9	70.9
30–60	20.7–154	39.1	46.5	36.3	23.8	27.3	22.1
0–30	0–20.7	13.0	14.8	11.4	5.9	7.8	7.0

DNA was isolated and centrifuged as described in Fig. 1, except that ^{32}P -labelled DNA was not coisolated with ^3H -labelled DNA. The DNAs were extracted from embryos grown without added nucleoside or with the addition of BUdR or TdR to $50 \mu\text{g ml}^{-1}$.

* $S_{20,w}$ values were determined by the relative sedimentation distance of freshly prepared ^3H -TdR-labelled $\phi 80$ DNA¹² cosedimented in each gradient.

† Molecular weight was calculated from $S_{20,w}$ values using the equations of Studier¹³. Native $\phi 80$ phage DNA has a calculated $S_{20,w}$ value of 33.4 based on a molecular weight of 28.6×10^6 (ref. 14).

Thus it seems that the ^{32}P -labelled nuclear DNA isolated from early stage sea urchin embryos allowed to develop in the presence of BUdR contains an increased proportion of molecules in the 30–60S range, compared with DNA isolated from embryos grown in the absence of BUdR or in the presence of TdR. This shift in the size distribution of DNA could result from at least one of several mechanisms: (1) certain sequences in higher molecular weight DNA synthesised in the presence of BUdR may be sensitive to excision *in vivo* or breakage during the isolation of the DNA; (2) BUdR may induce the amplification of some episomal DNA; (3) certain newly synthesised DNA molecules may not be able to integrate into bulk DNA when substituted with BUdR. Though the data presented do not distinguish between the above possibilities, certain indirect evidence may favour the latter mechanism.

(1) Birnboim *et al.*⁶ have reported the presence of long (greater than 4S) pyrimidine stretches which remain after hydrolysis of HeLa cell DNA in the presence of formic acid and diphenylamine. If the incorporation of BUdR renders DNA more sensitive to excision *in vivo* or breakage during isolation, one might expect incorporation of BUdR to change the size distribution of such long pyrimidine lengths. A comparison of ^{32}P -labelled pyrimidine runs obtained from DNAs isolated from embryos grown in the presence or absence of BUdR demonstrated no difference in length distributions (S.T.C., unpublished observation). This might be taken as evidence against excision or breakage of BUdR-substituted DNA within long pyrimidine runs, though such an occurrence in a small proportion of the pyrimidine lengths or in short oligopyrimidines would not be detected.

(2) Kotzin and Baker³ reported that 8–15S (alkaline sucrose gradient) newly synthesised sea urchin DNA (apparently occurring as a normal intermediate in DNA synthesis⁷), pulse-labelled with ^3H -BUdR, could not be chased into higher molecular weight DNA with unlabelled BUdR during a 20 min chase period. These experiments suggest that a portion of newly synthesised short DNA pieces either cannot integrate with existing higher molecular weight DNA, or the rate of integration is retarded by the BUdR. In the results shown in Fig. 1 and Table 1, exposure of embryos to BUdR over a long period results in accumulation of pieces longer than the 8–15S size range seen at the end of the relatively short pulse-chase period previously described³. Thus, it seems possible that BUdR retards rather than prevents the integration of short pieces of certain nuclear DNAs. The 30–60S pieces accumulated in embryos continuously grown in BUdR may, however, represent a maximum size beyond which certain DNA sequences cannot increase by acquiring smaller DNA pieces or by integrating into existing longer pieces.

There is some support for the possibility that particular nuclear DNA sequences might incorporate BUdR preferentially. Walther *et al.*⁸ report that in the developing pancreas there is a low molecular weight, double-stranded, nuclear DNA fraction (detected with ^3H -thymidine) which normally does not incorporate into higher molecular weight DNA. This DNA fraction preferentially incorporates BUdR relative to bulk DNA¹. Reassociation studies demonstrate that this 15–25S fraction of DNA is not a random sample of chromosomal sequences and does not contain highly repetitive sequences¹.

It is perhaps interesting to note that the size of the DNA molecules which seem to be affected by exposure to BUdR during replication (30–60S in the work reported here) are within the size range reported for the replicons of several higher cell types^{9,10}. This suggests a possible mechanism for the selective effect of BUdR on certain specialised functions associated with differentiation. Substitution of BUdR for thymidine could preferentially prevent or retard integration, into higher molecular weight DNA, of those replicons containing sequences coding for or regulating products related to specialised functions associated with differentiation. If such integration is necessary for regulation of gene expression it would be possible for this thymidine analogue to inhibit differentiation without affecting cell viability and proliferation.

This work was supported by a grant from the National Institutes of Health, US Public Health Service. S.T.C. is a predoctoral trainee of the US Public Health Service.

ROBERT F. BAKER

STEVEN T. CASE

Department of Biological Sciences,
University of Southern California,
Los Angeles, California, 90007

Received January 28, 1974.

- Rutter, W. J., Pictet, R. L., and Morris, P. W., *A. Rev. Biochem.*, **42**, 601–646 (1973).
- Goncharoff, M., and Mazia, D., *Expl Cell Res.*, **46**, 315–327 (1967).
- Kotzin, B. L., and Baker, R. F., *J. Cell Biol.*, **55**, 74–81 (1972).
- Piko, L., Tyler, A., and Vinograd, J., *Biol. Bull.*, **132**, 68–90 (1967).
- Piko, L., *Am. Zool.*, **9**, 1118 (1969).
- Birnboim, H. C., Mitchel, E. J., and Straus, N. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2189–2192 (1973).
- Baker, R. F., *Biochem. biophys. Res. Commun.*, **43**, 1415–1420 (1971).
- Walther, B. T., David, J. D., Levine, S., Pictet, R., and Rutter, W. J., *Fedn Proc.*, **30**, 1128 (1971).
- Callan, H. G., *Proc. R. Soc. B*, **181**, 19–41 (1972).
- Taylor, J. H., Adams, A. G., and Kurek, M.P., *Chromosoma*, **41**, 361–384 (1973).
- Tyler, A., *Biol. Bull.*, **104**, 224–229 (1953).
- Thomas, jun., C. A., and Abelson, J., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. I. and Davies, D. R.), 553–561 (Harper and Row, New York, 1966).
- Studier, F. W., *J. molec. Biol.*, **11**, 373–390 (1965).
- Davidson, N., and Szybalski, W., in *The Bacteriophage Lambda* (edit. by Hershey, A. D.), 48 (Cold Spring Harbor Laboratory, New York, 1971).

Are 'N bands' selective staining of specific heterochromatin?

By *in situ* DNA-RNA-hybridisation and the association patterns in meiotic and mitotic chromosomes, the nucleolus organiser regions (NOR) have been localised on specific chromosomal segments of some mammalian species^{1–4}. Matsui and Sasaki⁵ described a staining technique ('N bands') which was assumed to stain the NOR of mammalian chromosomes with high specificity.

This assumption was based on the following findings. First, the satellites of human acrocentric chromosomes are exclusively stained but not the centromeres, short arms or stalks of satellites. From their interpretation of the literature^{1,2}, they believe that the NOR is located in the satellites of human D and G group chromosomes. Second, N bands appear in the secondary constriction of the rat kangaroo (*Potorous tridactylis*) X chromosome to which the NOR is assigned^{6,7}. Third, N bands are visible in the presumed nucleolus organiser regions of several other species (*Rattus norvegicus*, *Crisetulus griseus*, *Muntiacus muntiac*, *Macaca fascicularis* and *Mus musculus*).

We have checked these hypotheses using essentially the same technique⁵ with chromosome preparations of human lymphocytes, fibroblasts of the vole species *Microtus oeconomus* and *Microtus ochrogaster*, and the dormouse *Eliomys quercinus*.

In accordance with the results of Matsui and Sasaki, on human metaphase plates we found distinctive red spots on the satellites of acrocentric chromosomes (Fig. 1). Yet, not all satellites stain with this technique as should be the case if this technique gives a differential staining of the NOR. In contrast to the statements of these authors we observed an additional differential staining of the short arm of one D group chromosomes (Fig. 1). No staining of the stalks of satellites could be achieved in all metaphases analysed.

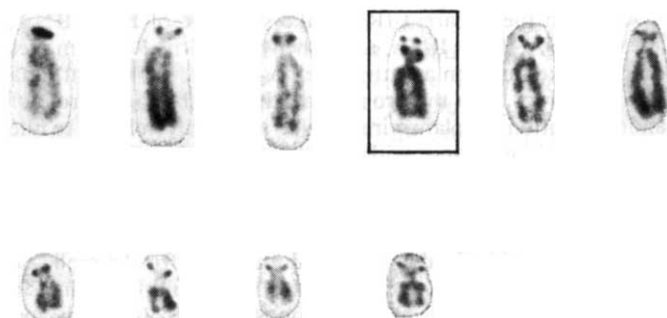


Fig. 1 D and G group chromosomes of a human lymphocyte in metaphase with N bands. Note the polymorphism of the N bands on the short arm and satellites in the marked D chromosome.

As the nucleolus organiser regions in human chromosomes have been assigned to the secondary constrictions of the acrocentric chromosomes^{1,2,4}, it has to be concluded that the N-band technique does not give a differential staining of the human NOR.

In metaphase chromosomes of *M. oeconomus*¹³ N bands were visible at the short arms of the acrocentric chromosomes 13 and 14. The unpublished results of W. Engel, H. Hoehn and W. Krone have shown that the short arm and the secondary constriction of the long arm of chromosome 14 are involved in 'satellite associations'.

Accordingly, in the long arm of chromosome 14 the NOR shows no N band and the N band of chromosome 13 is not correspondent to a NOR. These findings are evidence that the N-band positive sites do not coincide with NORs also in this species. We also treated metaphase plates of *M. ochrogaster* and *Eliomys quercinus* with the N band technique. It was not possible to detect any N bands in either species. As these species are expected to possess NOR, it is obvious that N bands do not represent a differential staining of the NOR.

In many of the species investigated, heterochromatic regions are located adjacent to the NORs. This is true for man⁹, mouse^{12,11}, Indian muntjac^{1,5}, and the short arm of chromosome 14 in *M. oeconomus*, for example. In a species

without heterochromatin (*Eliomys quercinus*, our unpublished results) no N bands can be found. Therefore we conclude that N bands correspond to a specific kind of heterochromatin which is situated adjacent to the NOR in most instances.

We have circumstantial evidence¹⁰ that the production of G bands is determined by qualitative differences of non-histone proteins along the chromosomes. Matsui and Sasaki⁵ give evidence that the specific nonhistone proteins are stained with the N-band technique. Therefore one may assume that N bands reflect nonhistone proteins corresponding to a special kind of heterochromatin. Though N bands apparently do not stain the NORs, they are useful markers in human cytogenetics like other special staining methods.

J. F. is in receipt of a trainee fellowship of the Deutsche Forschungsgemeinschaft.

JOACHIM FAUST
WALTHER VOGEL

Institut für Humangenetik und
Anthropologie der Universität
78 Freiburg i. Br.
Albertstr. 11, German Federal Republic

Received January 22, 1974.

- ¹ Henderson, A. S., Warburton, P., and Atwood, K. C., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 3394-3398 (1972).
- ² Bross, K., and Krone, W., *Humangenetik*, **14**, 137-141 (1972).
- ³ Comings, D. E., *Expl Cell Res.*, **67**, 441-460 (1971).
- ⁴ Ohno, S., Trujillo, J. M., Kaplan, W. D., and Kinoshita, R., *Lancet*, **ii**, 123-126 (1961).
- ⁵ Matsui, S.-J., and Sasaki, M., *Nature*, **246**, 148-150 (1973).
- ⁶ Hsu, T. C., Brinkley, B. R., and Arrighi, F. E., *Chromosoma*, **23**, 137-153 (1967).
- ⁷ Ohnuki, Y., Olson, R. S., and Rounds, D. E., *Jap. J. Genet.*, **47**, 133 (1972).
- ⁸ Hsu, T. C., and Arrighi, F., *Chromosoma*, **34**, 243-253 (1971).
- ⁹ *Paris Conf. (1971). Standardization Human Cytogenet., Birth Defects (original article Series), VIII*, 7 (The National Foundation, New York, 1972).
- ¹⁰ Vogel, W., Faust, J., Schmid, M., and Siebers, J.-W., *Humangenetik*, **21**, 227-236 (1974).
- ¹¹ Levan, A., Hsu, T. C., and Stich, H. F., *Hereditas*, **48**, 677-687 (1962).
- ¹² Hsu, T. C., Cooper, J. E., Mace, M. L., jun., and Brinkley, B. R., *Chromosoma*, **34**, 73-87 (1971).
- ¹³ *An Atlas of Mammalian Chromosomes* (edit. by Hsu, T. C., and Benirschke, K.) (Springer-Verlag, Berlin, Heidelberg and New York, 1967).

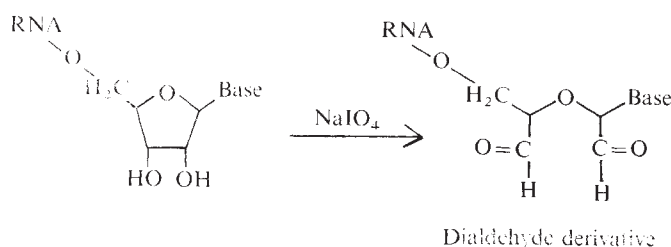
Messenger and aminoacylation functions of brome mosaic virus RNA after chemical modification of 3' terminus

THE ability of RNA from several plant viruses to bind a specific amino acid in a manner like tRNA is now well established. All members of the tymovirus group so far examined contain RNA which can bind valine¹⁻³; all members of the bromovirus group bind tyrosine (ref. 4 and T.C.H., in preparation), and tobacco mosaic virus (TMV) RNA binds histidine⁵. The ubiquity of the reaction, and the similarity of the esterification to that of aminoacylation of tRNA indicates that a significant biological function for the property exists. A tRNA-like function such as that of a virus-specific tRNA, or a mis-sense tRNA seems plausible. Although there is evidence that turnip yellow mosaic virus (TYMV) RNA, or a fragment containing the 3' terminus of TYMV RNA, may donate valine to nascent polypeptides⁶, it has been established that tyrosine, bound to brome mosaic virus (BMV) RNA, is not donated to peptidyl material in a cell-free system from wheat embryo when directed by viral, plant, or synthetic messenger RNA (ref. 7).

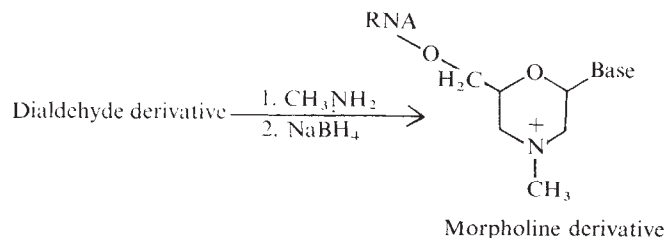
Of the several viral RNAs capable of aminoacylation, only BMV RNA has been shown to function as an efficient messenger.

capable of directing the synthesis of authentic viral proteins in a cell-free system⁸. Therefore, with BMV RNA, a unique opportunity exists for determining the inter-relationship between aminoacylation activity and messenger function of viral RNA. A useful approach to understanding this inter-relationship is to test retention of these activities following chemical modification of the viral RNA. A definitive modification should be that of oxidation of the 3' terminus as this is known to prevent aminoacylation of tRNA (ref. 9).

Two procedures were adopted for RNA modification¹⁰. First, the 3' end of BMV RNA was oxidised to the dialdehyde derivative with periodate according to the reaction:



It is known, however, that the oxidised 3' end of the RNA can be removed by an amine-catalysed β -elimination reaction¹¹, and therefore, the possibility existed that under our *in vitro* reaction conditions replacement of the terminal pA_{OH} could occur, thereby restoring the RNA to its original form. To prevent such repair of the 3' terminus the second modification procedure used was the preparation of the morpholine derivative according to the reaction:



Both modification procedures resulted in the loss of the ability of the BMV RNA to accept an amino acid but no quantitative difference in messenger function was observed (Table 1).

To determine whether qualitative differences exist in the pattern of proteins synthesised under the direction of the modified RNA, the products synthesised *in vitro* were subjected to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. Protein synthesised using unmodified BMV RNA (comprised of components 1,2,3, and 4) (ref. 12) as messenger consists mostly of BMV coat protein, together with a small amount of a protein designated protein 3a and some uncompleted proteins (Fig. 1a). The electrophoretic profiles of proteins synthesised under the direction of the dialdehyde derivative, and of the morpholine derivative, appear in Fig. 1b and c, respectively. The pattern of radioactivity obtained for products of the reaction directed by the dialdehyde derivative (Fig. 1b) was identical to that of the unmodified BMV RNA products (Fig. 1a), confirming that normal products can be synthesised by viral RNA lacking an aminoacylatable 3' terminus. Analysis of the products of the reaction directed by the morpholine derivative (Fig. 1c) also showed the presence of coat protein and protein 3a. In this case, however, a relatively higher proportion of protein 3a, and a correspondingly lower proportion of coat protein was synthesised. The reason for this difference is unknown. A likely possibility is that configurational changes

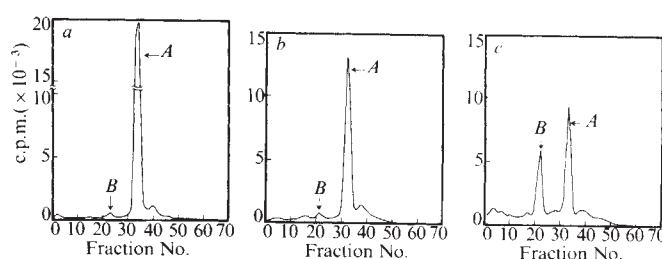


Fig. 1 Polyacrylamide gel patterns of products synthesised *in vitro* under the direction of modified and unmodified BMV RNA. Conditions for *in vitro* protein synthesis and gel electrophoresis were as previously described⁸. ¹⁴C-leucine was used as label. The products synthesised under the direction of unmodified RNA (control), the dialdehyde derivative of the RNA, and the morpholine derivative of the RNA are shown in (a), (b), and (c), respectively. Electrophoresis was from left to right. A, Coat protein; B, protein 3a.

occurred in the BMV RNA molecules after methylamine and sodium borohydride treatment, causing alterations in the affinities of ribosomes for the different cistrons. While this in itself is an interesting finding, the fact that both products synthesised under the direction of the modified RNAs are similar to proteins obtained using native BMV RNA as messenger strongly suggests that aminoacylation of BMV RNA is not an obligatory step for translation of this viral RNA.

Although the apparent tRNA-like structure of BMV RNA, TYMV RNA, TMV RNA, and other plant virus RNAs makes a tRNA-like function an attractive possibility, the present data greatly detract from the proposition that the biological role of aminoacylation of plant virus RNA is directly related to the donation of an amino acid to a viral peptide. The present results do not eliminate the possibility of such a role as a helpful, but not obligatory, step in viral growth. Nevertheless, it now seems more likely that binding of an amino acid to the virus RNA could be involved as a recognition signal for the replicase as proposed by Litvak and coworkers¹³, or could function as a regulatory system such as that proposed by Kolakofsky and Weissmann for phage Q β (ref. 14).

Table 1 Effect of modification of 3' terminus of BMV RNA on its tyrosine binding ability and on its activity in stimulating amino acid incorporation.

RNA added	Tyrosine binding (c.p.m.)	Amino acid incorporation (c.p.m.)
Control	10,239	13,641
Periodate derivative	254	13,000
Morpholine derivative	495	13,882

BMV RNA was modified according to the procedures described by Fahnestock and Nomura¹⁰ for modification of *Escherichia coli* 5S rRNA. For preparing the control RNA, all the manipulations for periodate oxidation and methylamine-borohydride derivatisation were carried out except that the modifying agents were omitted. Conditions for BMV RNA aminoacylation were as previously described⁸, each reaction (0.1 ml) containing 15 μ g of RNA. A 20 μ l sample was assayed for ³H-tyrosine binding to virus RNA after 30 min incubation at 30°C. Amino acid incorporation conditions were as previously described⁸. Each incorporation mixture (175 μ l) contained 30 μ g of RNA and 0.5 μ Ci of a mixture of ¹⁴C-labelled alanine, arginine, leucine, and lysine. Aliquots of 50 μ l were counted to determine amino acid incorporation into protein.

We thank Professor H. L. Shands of the Agronomy Department of the University of Wisconsin for providing us with wheat seeds, Mr Timothy Johnson for discussions, and Janice Metcalf for technical assistance. This work was supported by the Institute of Allergy and Infectious Diseases of the US National Institutes of Health and by the Biological Division of the US

Atomic Energy Commission.

Biophysics Laboratory, Graduate School,

Biochemistry Department,

Horticulture Department, College of
Agricultural and Life Sciences,
University of Wisconsin,
Madison, Wisconsin 53706

D. S. SHIH

PAUL KAESBERG

T. C. HALL

Received October 31, 1973.

- ¹ Litvak, S., Carré, D. S., and Chapeville, F., *FEBS lett.*, **11**, 316 (1970).
- ² Pinck, M., Yot, P., Chapeville, F., and Duranton, H. M., *Nature*, **226**, 954 (1970).
- ³ Pinck, M., Chan, S.-K., Genevaux, M., Hirth, L., and Duranton, H., *Biochimie*, **54**, 1093 (1972).
- ⁴ Hall, T. C., Shih, D. S., and Kaesberg, P., *Biochem. J.*, **129**, 969 (1972).
- ⁵ Oberg, B., and Philipson, L., *Biochem. biophys. Res. Commun.*, **48**, 927 (1972).
- ⁶ Haenni, A. L., Prochiantz, A., Bernard, O., and Chapeville, F., *Nature new Biol.*, **241**, 166 (1973).
- ⁷ Chen, J. M., and Hall, T. C., *Biochemistry* **12**, 4570 (1973).
- ⁸ Shih, D. S., and Kaesberg, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1799 (1973).
- ⁹ Deutscher, M. P., in *Progress in Nucleic Acid Research and Molecular Biology* (edit. by Davidson, J. N., and Cohn, W. E.) **13**, 51 (Academic Press, New York, 1973).
- ¹⁰ Fahnestock, S. R., and Nomura, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 363 (1972).
- ¹¹ Neu, H. C. and Heppel, L. A., *J. biol. Chem.*, **239**, 2927 (1964).
- ¹² Lane, L. C., and Kaesberg, P., *Nature new Biol.*, **232**, 40 (1971).
- ¹³ Litvak, S., Tarragó, A., Tarragó-Litvak, L., and Allende, J. E., *Nature new Biol.*, **241**, 88 (1973).
- ¹⁴ Kolakofsky, D., and Weissmann, C., *Nature new Biol.*, **231**, 42 (1971).

Virus-induced cell fusion enhanced by phytohaemagglutinin

ONCOGENIC transformation by viruses or chemicals¹⁻⁵, treatment with proteolytic enzymes^{1,6} and infection with nononcogenic enveloped viruses^{7,8}, produce cell surface alterations that increase cellular susceptibility to agglutination by plant lectins. More recently, lectins have been used to modify the replication and cytopathogenicity of enveloped viruses grown in normal cells^{9,10}. It has been shown that treatment of cells with con A inhibits paramyxovirus-induced cell fusion.¹¹ In contrast, the results reported here indicate that another plant lectin, phytohaemagglutinin (PHA), can enhance the fusion of cells infected with the paramyxovirus Newcastle disease virus (NDV).

Freeze-dried PHA (Wellcome Reagents Ltd., Beckenham, Kent) was reconstituted in phosphate-buffered saline (PBS) and cells were treated with 1.0 ml diluted in maintenance medium at the concentrations described below. The properties of the NDV strains, cell culture methods, infection of cell cultures and measurement of the extent of cell fusion have been described¹²⁻¹⁴. All cell cultures were grown on coverslips in Leighton tubes at 37° C.

To demonstrate the effect of different concentrations of PHA on NDV-induced cell fusion from within¹⁵, virus was removed 1 h after infection and replaced with 1.0 ml of medium containing different dilutions of PHA, incubated for a further 8 h at 37° C in the presence of PHA, finally fixed and stained and the extent of cell fusion measured. Fusion of BHK 21 cells infected with NDV strain Herts was enhanced in the presence of PHA (Fig. 1). Incubation of PHA with 0.5 M N-acetyl-galactosamine, a haptenic inhibitor of PHA, for 1 h at 37° C before addition to infected cell cultures eliminated its ability to enhance fusion

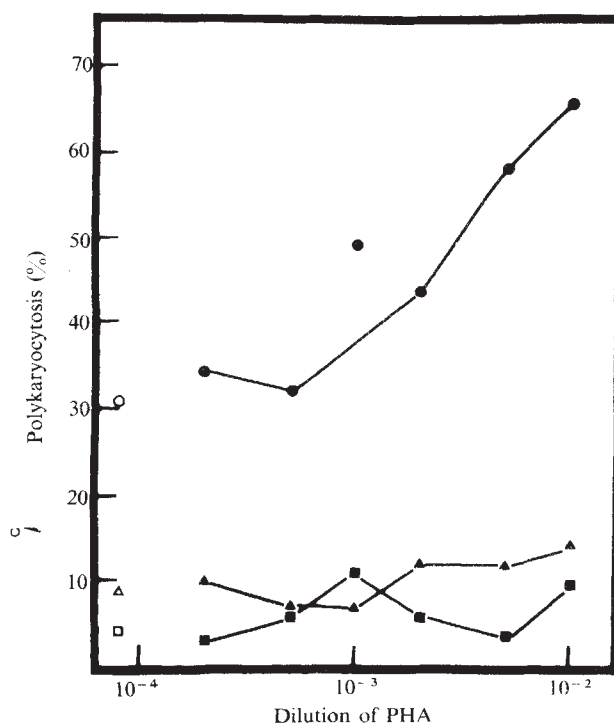


Fig. 1 Effect of different concentrations of PHA on fusion of BHK-21 cells infected with 25 EID₅₀/cell NDV-Herts (●), 50 EID₅₀/cell NDV-Queensland (■), and uninfected control cells (▲). At 1 h after infection, virus was replaced with PHA diluted in maintenance medium. Polykaryocytosis was estimated 8 h after infection. O, □, △, Fusion in the absence of PHA.

from within by strain Herts. PHA had no effect, however, on the fusion of cells infected with NDV strain Queensland (a non-fusing strain) or uninfected control cells. The time of addition of PHA after infection was important in determining the degree of enhancement of cell fusion (Fig. 2). The length of time for which PHA is in contact with the cells is not important, however, as similar enhancement of fusion from within was found after exposure to PHA for 90 min or 8 h with PHA added 1 h after infection. It is more likely that the decrease in enhancement is related to the progression of virus replication and fusion (Fig. 2), that is, once a fusion event has taken place there can be no enhancement of that event.

Fusion from without (ref. 15) was also enhanced by pretreatment with PHA. Maximum enhancement was recorded if cell cultures were treated with PHA (10⁻² dilution) for 1.5 h or more before infection. Such treatment before infection with 1600 EID₅₀/cell NDV-Herts induced 57.5% polykaryocytosis compared to 37.2% in untreated and 4.1% in uninfected cell cultures. Experiments using PHA-Sepharose 4B conjugate gave similar results and showed that it was not necessary for PHA to enter the cell to cause enhancement. Cells were pretreated for 1.5 h with PHA-Sepharose, which had been washed extensively to remove any free PHA and diluted to give similar activity (as titrated by haemagglutination) to the unbound PHA used, and then infected with 1600 EID₅₀/cell NDV-Herts. Three hours after infection, cells treated with PHA-Sepharose showed 57.5% polykaryocytosis compared to 37.2% in infected, untreated cells and 3.9% in uninfected cells.

The results presented here indicate that PHA has a marked enhancing effect on cell fusion by NDV and that PHA acts rapidly on a prefusion event and does not require the presence of virus products at the cell surface for the initial interaction. The results obtained with PHA-Sepharose indicate that, as with lymphocyte

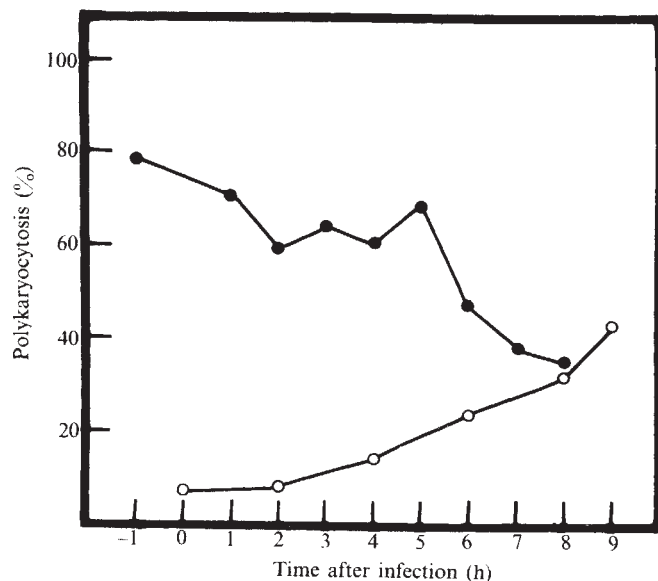


Fig. 2 Effect of PHA (10^{-3} dilution) added at different times on cell fusion of BHK-21 cells at 9 h after infection with 25 EID₅₀/cell NDV-Herts (●). Fusion in untreated cells at different times after infection with NDV-Herts is also shown (○).

activation¹⁶, PHA does not have to enter the cell to be effective.

Further work is required to determine the mode of action of PHA. It may be significant, however, that other studies have indicated that lysosome fragility follows stimulation of lymphocytes with PHA¹⁷ and we have previously suggested the possible role of lysosomal enzymes in virus-induced cell fusion¹⁸.

This work was aided by a grant from the National Cancer Institute of the United States, and a grant from the Agriculture Research Council. G.H. and H.W. were supported by the Agriculture Research Council. PHA-Sepharose was a gift from Dr K. H. Fantes, Wellcome Research Laboratories, Beckenham.

P. REEVE
G. HEWLETT
H. WATKINS

Department of Bacteriology,
University College Hospital Medical School,
London, WC1

D. J. ALEXANDER

Central Veterinary Laboratory,
New Haw,
Weybridge, Surrey

G. POSTE

Department of Experimental Pathology,
Roswell Park Memorial Institute
Buffalo, New York 14203

Received December 27, 1973.

- ¹ Inbar, M., and Sachs, L., *Proc. natn. Acad. Sci., U.S.A.*, **63**, 1418 (1969).
- ² Inbar, M., and Sachs, L., *Nature*, **223**, 710 (1969).
- ³ Aub, J. C., Sanford, B. H., and Cote, M. N., *Proc. natn. Acad. Sci., U.S.A.*, **54**, 396 (1965).
- ⁴ Burger, M. M., and Goldberg, A. R., *Proc. natn. Acad. Sci., U.S.A.*, **57**, 359 (1970).
- ⁵ Cline, M. J., and Livingston, D. C., *Nature new Biol.* **232**, 155 (1971).
- ⁶ Burger, M., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 994 (1969).
- ⁷ Becht, H., Rott, R., and Klenk, H-D., *J. gen. Virol.* **14**, 1 (1972).
- ⁸ Poste, G., Reeve, P., Alexander, D. J., and Terry, G., *J. gen. Virol.*, **17**, 81 (1972).
- ⁹ Rott, R., Becht, H., Klenk, H-D., and Scholtissek, C., *Z. Naturforsch.* **27**, 227 (1972).

- ¹⁰ Reeve, P., Poste, G., and Alexander, D. J., in *Negative Strand Viruses* (edit. by Mahy, B. W. J., and Barry, R.) (Academic Press, London, in the press).
- ¹¹ Poste, G., Alexander, D. J., Hewlett, G., and Reeve, P., *J. gen. Virol.* (in the press).
- ¹² Alexander, D. J., Reeve, P., and Allan, W. H., *Microbios*, **2**, 155 (1970).
- ¹³ Reeve, P., and Poste, G., *J. gen. Virol.*, **11**, 17 (1971).
- ¹⁴ Poste, G., Waterson, A. P., Terry, G., Alexander, D. J., and Reeve, P., *J. gen. Virol.*, **16**, 95 (1972).
- ¹⁵ Bratt, M. A., and Gallaher, W. R., *Proc. natn. Acad. Sci., U.S.A.*, **64**, 536 (1969).
- ¹⁶ Greaves, M. F., and Bauminger, S., *Nature new Biol.*, **235**, 67 (1972).
- ¹⁷ König, E., Brittinger, G., and Cohnen, G., *Nature new Biol.*, **244**, 247 (1973).
- ¹⁸ Reeve, P., Poste, G., Alexander, D. J., and Pope, G., *J. gen. Virol.*, **15**, 219 (1972).

Thymus involvement in female sexual maturation

It is now established that the thymus plays a critical role in a variety of immunological functions. Evidence that the thymus can also function as an endocrine gland has mainly been derived from immunological investigations^{1,2}. Because endocrine glands do not function independently, it is likely that the thymus is involved in a number of endocrine functions and some experimental data support such an assumption. Thus, hypothyroidism and persistence of the foetal zone in the adrenals is observed in congenitally athymic nude mice³ and degranulation of pituitary eosinophil cells occurred following neonatal thymectomy in mice⁴.

Here we present experimental evidence that the thymus also influences the maturation of female sexual function during a critical period of development.

Conventional female Charles River mice were surgically thymectomised or sham-operated when 2 or 10 d old. Mice thymectomised on day 2 were implanted on day 3 with 1-2 thymus glands obtained from male or female animals of the same age and other groups of 3 d old were injected intraperitoneally with 5×10^6 thymocytes from adult mice (2-3 months old). Among the parameters studied in the different experimental groups were vaginal opening time (VOT) (the day of complete vaginal opening with full differentiation of the external sexual structures); the sexual cycle (based on daily cytological studies of the vaginal contents), and the morphology of the ovary and uterus (based on gross inspection and conventional histology). The criterion for defining puberty was the vaginal opening time followed by the first oestrus appearing within 4 d.

On completion of the various experiments, all mice were killed for gross morphological and histological inspection of the sexual organs. Any animals found to be incompletely thymectomised were discarded.

It can be seen in Table 1 that there was a marked delay in VOT in animals that had been thymectomised on day 2 (VOT 50.5 ± 1.4 d) compared with the untreated (VOT 35.1 ± 0.8 d) and sham-operated mice (VOT 34.8 ± 0.9 d). It is especially noteworthy that when thymectomy was performed on day 10, there was no delay in VOT (35.4 ± 1.4 d). Grafting the thymus into mice 3 d old and thymectomised 1 d earlier, counteracted the delay in the VOT (37.8 ± 1.5). In contrast to an intact thymus, the administration of 5×10^6 thymocytes did not influence the delay in the VOT in thymectomised 2-d-old mice (47.8 ± 2.8 d).

The sexual cycle, as reflected by vaginal cytology, showed that irrespective of thymic presence or lack of it, once the vagina had opened, the first oestrus appeared in all animals within 4 d. The cycles were entirely normal during the 15 d of observation.

Morphological studies in neonatally thymectomised mice revealed that before the delayed vaginal opening occurs, the ovaries and uterus are very small and display the typical

Table 1 Delay in vaginal opening time in Charles River mice after neonatal thymectomy and restoration by early thymus grafting

Treatment	Number of animals	Vaginal opening time (d)				$\chi^2 \pm \text{s.e.m.}$
		< 40	> 40 < 55	> 55		
Controls (normal)	33	33	—	—		35.1 $\pm$ 0.8
Controls (sham-operated)	14	14	—	—		34.8 $\pm$ 0.9
Thymectomised on day 2	33	6	20	7		50.5 $\pm$ 1.4
Thymectomised on day 10	5	5	—	—		35.4 $\pm$ 1.4
Thymectomised on day 2; grafted with thymus on day 3*	11	8	2	1		37.8 $\pm$ 1.5
Thymectomised on day 2; injected with 5×10^6 thymocytes on day 3	8	1	5	2		47.8 $\pm$ 2.8

* Syngeneic thymus graft obtained from male or female 3-d-old Charles River mice.

histological pattern characteristic of prepubertal immaturity.

What we have said of the thymectomised mice is also true of congenitally athymic female nude mice, the behaviour of which prompted us to investigate the thymectomised animals. Nude mice have a thymic anlage in the foetus but on day 14 of pregnancy it is already abnormal. The animals have a scarcity of lymphocytes in the paracortical area of lymph nodes and fail to reject allogeneic skin grafts⁵.

Congenitally athymic female nude mice (*nu/nu* BALB/c) and their normal thymus-bearing female litter mates (BALB/c) were kept in specific pathogen-free (SPF) conditions until transfer to this laboratory. Some of the animals were bred and kept in germ-free conditions.

Data on athymic nude mice are given in Table 2. Vaginal opening time was also delayed in germ-free conditions.

The great difference in the size of the uterus and ovary between a 43-d-old athymic nude and a normal mouse is clearly shown in Fig. 1. Especially striking is the normalisation of these organs following thymus transplantation on day 2. By 10 d after VOT, the ovaries and uterus had already developed and presented an entirely normal appearance by increasing their residence times. Also that any appearance histologically.

To determine whether the thymus involvement in sexual maturation is dependent on its immunological function, newborn nude mice were injected intraperitoneally with 15×10^6 each of thymocytes (four animals); spleen cells (three animals); and lymph node cells (two animals) from adult BALB/c mice 3 months old.

Vaginal opening time was found to be delayed (VOT 49.1 ± 0.4 d). 1–2 weeks after VOT, the animals were transplanted with skin from C57 black mice. After 2 weeks, six out of nine nude mice injected with cells had rejected the skin grafts. Thus, it seems that whereas the injected lymphoid cells can establish cell-mediated immunity in the nudes, they cannot normalise sexual maturation.

Our findings indicate that the absence of the thymus in the early stages of life, as in Charles River mice thymectomised on day 2 and in congenitally athymic nude mice, causes a significant delay in puberty (as reflected in vaginal opening time and first oestrus) and a delay in the maturation of the ovaries and uterus. A few days after attainment of the VOT, a normal sexual cycle and normal uterus and ovary morphology are established. The fact that the same delay in sexual maturation occurred in germ-free nude mice rules out the likelihood that in these immunodeficient

animals, infection is involved in this phenomenon. Thymus grafts were able to speed up the onset of puberty provided they were implanted on the second or third day of life. Postponement of thymus implantation to day 10 no longer effected an acceleration of the sexual function. Furthermore, the injection of lymphoid cells into newborn nudes does not normalise sexual maturation. All these findings indicate a causal relationship between a functioning thymus and the normal development of sexual function in the female during the early stages of postnatal life.

Pfeiffer⁶, showed that the pattern of sexual functions is established during the perinatal period. The presence or absence of the testicular hormone determines the future male or female rhythm of gonadotrophin release by the hypophysis (tonic or cyclic pattern). Current concepts^{7,8} consider sexual hormones as at least partially responsible for the organisation of some of the central nervous system (CNS) control mechanisms which determine the activation of the sexual endocrine system in the adult.

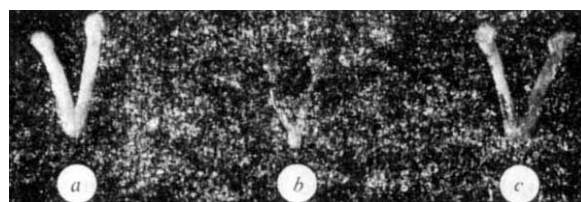


Fig. 1 Normalisation of sexual organ development by thymus. Gross appearance of uterus and ovaries of 43-d-old BALB/c mice (a), athymic nude mice (b), and athymic nude mice with a thymus graft (c). The thymus graft was made on animals 2 d old.

As the thymus has now been shown to influence the onset of the sexual functions, and as this effect is observed during the perinatal period, in which the CNS is being programmed, it is a reasonable assumption that the thymus is likewise involved in this process. The onset of puberty is a neuroendocrine phenomenon⁹; for this reason one can conceive of the thymus exerting its influence by the elaboration of hormones that could either act directly on the CNS or indirectly through endocrine glands. These hormonal influences of the thymus may be greatest during the perinatal period, when this organ is known to be more crucial than in later life.

Table 2 Delay in vaginal opening time in congenitally athymic nude mice (*nu/nu* BALB/c) and restoration by early thymus grafting

Treatment	Number of animals	Vaginal opening time (d)				$\chi^2 \pm \text{s.e.m.}$
		< 35	> 35 < 50	> 50		
Controls (normal BALB/c*)	55	55	—	—		31.3 $\pm$ 0.4
Athymic nude (conventional)	39	4	28	7		45.8 $\pm$ 0.9
Athymic nude (germ-free)	8	—	6	2		48.0 $\pm$ 1.6
Athymic nude grafted with thymus on day 2†	11	10	1	—		33.2 $\pm$ 0.9
Athymic nude grafted with thymus on day 10‡	7	1	5	1		46.9 $\pm$ 1.5
Athymic nude with thymocytes injected on day 2§	7	1	5	1		47.1 $\pm$ 1.6

* BALB/c mice; normal brothers of the nudes.

† Thymus from male and female 2-d-old BALB/c mice.

‡ Thymus from male or female 10-d-old BALB/c mice.

§ Thymocytes from male and female adult BALB/c mice 3 months old.

The delay in puberty in mice without thymus glands can provide an explanation for the persistence of the reticular zone of the adrenals in nude mice³ because this zone tends to disappear after puberty.

Nishizuka and Sakakura¹⁰⁻¹² have observed earlier ovary dysgenesis (lack of follicles and of corpora lutea) and hyperplasia of the interstitial cells in about 50% of neonatally thymectomised female mice. This work, however, was not concerned with sexual development, because, depending on the strain studied, the dysgenesis was not observable before 60-120 d of age, a time when puberty had already occurred and normal sexual cycles were well established. This ovarian dysgenesis was prevented by spleen or thymus grafts and by lymphoid cell injection given during the first days of life. These experiments were performed in animals maintained in conventional conditions. As the administration of lymphoid cells and thymus or spleen grafts (which can restore immune functions) prevented the ovarian dysgenesis, the results obtained by these authors could have been influenced by intercurrent infection and might reflect an early manifestation of wasting disease or express some lymphoid cell function. Whichever of these possibilities apply, this situation can hardly involve an endocrine thymic function.

It has been demonstrated that the thymus is involved in the complex endocrine mechanisms controlling sexual development during a critical period of life, presumably by an elaboration of hormonal factor, as immunocompetent cells can not compensate for thymus absence, as far as sexual maturation is concerned. Such thymus involvement in sexual function has been suspected earlier as reviewed by Hammar¹³. In as much as the thymus is involved in the onset of puberty, and thymus involution also begins at this time, this would seem to indicate that a feedback mechanism is in operation.

We thank Dr H. Hurni and Gisela Hammerli, Ciba-Geigy, Sisseln, for their help and observation in the germ-free animals and Anna Arcon for technical assistance. Sir Peter Medawar and Dr M. Landy criticised the manuscript. The congenitally athymic female nude mice (*nu/nu* BALB/c) and their normal thymus-bearing female littermates (BALB/c) were purchased from Dr C. W. Friis, GI. Bomholtgård, Ry, Denmark. This work was supported by a grant from the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung. H. B. is the recipient of an Argentine National Research Council fellowship.

H. O. BESEDOVSKY
E. SORKIN

Schweizerisches Forschungsinstitut
Medizinische Abteilung,
7270 Davos, Switzerland

Received November 26, 1973; revised February 4, 1974.

- 1 *Thymic Hormones* (edit. by Luckey, T. D.) (Urban and Schwarzenberg, München, Berlin and Wien, 1973).
- 2 Stutman, W., and Good, R. A., in *Contemporary Topics in Immunobiology* (edit. by Davies, A. J. S., and Carter, R. L.) 2, 299 (Plenum Press, New York and London, 1973).
- 3 Pierpaoli, W., and Sorkin, E., *Nature new Biol.*, **238**, 282 (1972).
- 4 Pierpaoli, W., Fabris, N., and Sorkin, E., in *Hormones and the Immune Response*, Ciba Foundation Study Group No. 36 (edit. by Wolstenholme, G. E., and Knight, Julie) 126 (J. & A. Churchill, London, 1970).
- 5 Pantelouris, E. M., *Immunology*, **20**, 247 (1971).
- 6 Pfeiffer, C. A., *Am. J. Anat.*, **58**, 195 (1936).
- 7 Harris, G. W., *Endocrinology*, **75**, 627 (1964).
- 8 Levine S., and Mullins, R. F., jun., *Science*, **152**, 1585 (1966).
- 9 Donovan, B. T., and Van der Werff ten Bosch, J., *Physiology of Puberty* (Williams and Wilkins, Baltimore, 1965).
- 10 Nishizuka, Y., and Sakakura, T., *Science*, **166**, 753 (1969).
- 11 Nishizuka, Y., and Sakakura, T., *Endocrinology*, **89**, 886 (1971).
- 12 Nishizuka, Y., and Sakakura, T., *Endocrinology*, **89**, 902 (1971).
- 13 Hammar, J. A., *Die normal-morphologische Thymusforschung. Analyse und Synthese* (Barth, J. H., Leipzig, 1936).

Evidence for non-identity of T killer and T helper cells sensitised to allogeneic cell antigens

BONE marrow-derived lymphocytes (B cells) can be triggered for antibody synthesis by a stimulus from immunocompetent allogeneic¹ or xenogeneic² lymphoid cells. The stimulus was shown to act directly on the B cell, since a response may occur in the absence of syngeneic thymus-derived lymphocytes (T cells)¹ and is possible even to a hapten³. This observation has led to the hypothesis that cooperating T cells (T-helper) are identical to cytotoxic T cells (T-killer) and act on B cells by surveying them for immunogen bound to their cell surface³. The T cells recognise the cell-bound immunogen and emit a sublethal signal to the B cells, triggering them to produce antibody. But the question arises, whether this is the way triggering of B cells usually occurs in the immune response. Recent experiments have shown that T cells sensitised to foreign erythrocyte antigens are able to cooperate with B cells to induce humoral antibody synthesis, but are not able to perform cytotoxic lysis of erythrocyte target cells^{4,5}. T helper cells therefore need not be cytotoxic to the sensitising antigen to trigger B cells. This finding led to more extensive experiments concerning the question of whether T killer and T helper functions are performed by the same cell or not.

I shall show here that T helper and T killer cells sensitised to the same immunogen are not likely to be identical, because they are found to be mutually exclusive under certain immunisation conditions.

The DBA/2J mastocytoma P185 injected into C57BL/6J mice has been shown to induce cytotoxic effector cells as well as humoral antibody synthesis^{6,7}. For both of these responses, T cells are necessary: T killer cells act as cytotoxic effectors⁶ and T helper cells trigger B cells for antibody synthesis. This model therefore seemed to be suitable to explore the relation of T helper and T killer cells. I set up experiments to find immunisation procedures which favour the appearance of either T killer or T helper activity in the spleen. Since it had previously been shown that treatment of erythrocytes with formaldehyde or glutaraldehyde inhibits the humoral immune response to such treated immunogen, without affecting the priming of T helper cells⁸, P185 cells were fixed in the same way. P185 cells treated with formaldehyde or glutaraldehyde (F-P185 and G-P185) were injected into C57BL/6J mice and their spleens assayed for cytotoxicity after 9 d. It is seen from Table 1 that F-P185 and G-P185 cells are not able to induce cytotoxicity in C57BL/6J splens, while normal P185 cells do so.

To find out whether one or the other of such immunised spleen cell preparations contain T helper cells, the same spleen cell preparations were incubated in Mishell Dutton type cultures⁹ together with trinitrophenyl-coupled¹⁰ P185 cells (TNP-P185) as antigen. Table 1 shows that while F-P185 and G-P185 primed spleens vigorously stimulate the TNP response, cytotoxic P185 primed spleens do not. This result could have various explanations: it may be that P185 primed spleen cells are not able to respond to *in vitro* antigen stimulation. But in other experiments it was shown that P185 primed spleen cells respond as well as, or even better than, normal or F-P185 primed spleen cells to sheep red blood cell antigens *in vitro* (unpublished results).

Another possibility could be that P185 primed spleen cells do contain P185 specific T helper cells but these helper cells do not display their activity, maybe because they are not at the proper concentration in these spleens. To check this, cytotoxic P185 primed spleen cells were mixed in varying proportions with normal spleen cells and assayed for carrier priming. Table 2 demonstrates that also under these conditions no helper function can be detected in cytotoxic spleens. Cytotoxic spleens therefore seem to contain no

Table 1 Induction of cell-mediated cytotoxicity and helper activity by normal and fixed P815 cells

<i>In vivo</i> immunogen	<i>In vitro</i> cytotoxicity assay		<i>In vitro</i> helper activity assay	
	% cytotoxicity at 1 h	2.5 h	10 ⁶ TNP- P815 cells per culture	P.F.C. per 10 ⁶ on TNP-BRBC
Experiment 1:				
10 ⁷ P815	15	33	—	69
10 ⁷ P815	—	—	+	3
10 ⁷ F-P815	1	1	—	80
10 ⁷ F-P815	—	—	+	540
10 ⁷ G' 0.25 P815	0	1	—	42
10 ⁷ G' 0.25 P815	—	—	+	121
10 ⁷ G' 0.025 P815	0	0	—	62
10 ⁷ G' 0.025 P815	—	—	+	248
Experiment 2:	2 h	4 h		
—	—	—	—	16
—	—	—	+	8
10 ⁷ P815	33	69	—	44
10 ⁷ P815	—	—	+	1
2 × 10 ⁷ F-P815	0	0	—	34
2 × 10 ⁷ F-P815	—	—	+	196

C57BL/6J mice were injected with varying doses of normal and fixed P815 cells intraperitoneally (i.p.) on day -11. DBA-2 mastocytoma cells P815 were grown in Dulbecco's modified MEM supplemented with 10% calf serum and fixed with 1.5% formaldehyde, 0.25% or 0.025% glutaraldehyde exactly as previously described for erythrocytes⁸. *In vitro* cytotoxicity assay: spleen cells of immunised mice were assayed for cytotoxicity using ⁵¹Cr-labelled P815 target cells as described previously^{5,6}. Attacker to target cell ratio was 100:1. Percentage cytotoxicity (over spontaneous release) is calculated from the ratio of released counts to that released after three times freeze thawing⁵. *In vitro* helper activity assay: spleen cells were incubated in Mishell Dutton type cultures⁹ with 10⁶ TNP-P815 cells per culture. TNP-P815 cells were prepared as described for erythrocytes by Rittenberg and Pratt¹⁰ and subsequently irradiated with 5,000 r. The TNP response was assayed on day 5 on TNP-BRBC^{9,10}.

helper activity as assayed with TNP-P815 antigen *in vitro*. This finding is somewhat surprising since it had previously been shown that in mice injected with P815 cells antibody production can be detected⁷. T helper cells therefore should at least at some time be operative during the *in vivo* immune response to P815.

Another possibility could be that the helper cells in

Table 2 Failure to detect carrier priming in mixtures of P815 primed spleen cells and normal spleen cells

Spleen cells primed with	dose per culture	10 ⁷ Normal spleen cells per culture	10 ⁶ TNP- P815 per culture	P.F.C. per 10 ⁶ on TNP-BRBC
—	—	+	—	26
—	—	+	+	32
F-P815	10 ⁷	—	—	36
F-P815	10 ⁷	—	+	352
P815	10 ⁷	—	—	49
P815	10 ⁷	—	+	48
P815	5 × 10 ⁶	+	—	31
P815	5 × 10 ⁶	+	+	33
P815	10 ⁶	+	—	45
P815	10 ⁶	+	+	8
P815	2.5 × 10 ⁵	+	—	23
P815	2.5 × 10 ⁵	+	+	3
P815	5 × 10 ⁴	+	—	73
P815	5 × 10 ⁴	+	+	3
P815	5 × 10 ³	+	—	35
P815	5 × 10 ³	+	+	27

C57BL/6J mice were immunised with 10⁷ F-P815 or 10⁷ P815 cells i.p. on day -11. Spleen cells were collected and tested for cytotoxicity. Spleens primed with P815 showed 62% cytotoxicity at *t* = 4 h at an attacker to target cell ratio of 100:1, spleen cells primed with F-P815 had no detectable cytotoxicity. Aliquots of spleen cells were cultured with TNP-P815 cells as antigen. P815 primed spleen cells were also mixed in various ratios with normal spleen cells. The plaque assay was done on day 5.

cytotoxic spleens are not detected, because their activity is suppressed by a population of suppressor cells like those described by Jacobson and Herzenberg¹¹. To find out, cytotoxic spleens and carrier-primed spleens were mixed and assayed for helper activity. It is seen in Table 3, experiment 1, that cells from cytotoxic spleens suppress the helper activity of F-P815 primed spleens, either partially (Table 3) or completely (unpublished results). It is therefore not surprising that helper activity cannot be detected in cytotoxic spleens using this assay. The question arises: why do live P815 cells prime for both killer and suppressor activity but not for helper activity, while fixed P815 cells prime only for helper but not for killer and suppressor activity? Though it is tempting to speculate that the cytotoxic T cells are the cells which suppress the function of the helper cells, this has to be shown by further experiments.

Carrier priming for erythrocyte antigens is known to be especially effective with low doses of antigen^{8,12}. Since fixation of P815 cells with formaldehyde destroys about 95% of

Table 3 Inhibition of helper activity by cytotoxic spleen cells and anti-θ serum treatment

Spleen cells per culture	10 ⁶ TNP-P815 per culture	P.F.C. per 10 ⁶ on TNP-BRBC
Experiment 1:		
10 ⁷ normal	—	12
10 ⁷ normal	+	19
10 ⁷ P815 immunised	—	8
10 ⁷ P815 immunised	+	12
10 ⁷ F-P815 immunised	—	19
10 ⁷ F-P815 immunised	+	350
5 × 10 ⁶ P815 immunised +	—	25
5 × 10 ⁶ F-P815 immunised	+	63
Experiment 2:		
normal	—	<1
normal	+	<1
F-P815 immune untreated	—	4
F-P815 immune untreated	+	134
F-P815 immune + anti-θ + C'	+	<1
F-P815 immune + C'	+	132

Experiment 1: C57BL/6J mice were primed with 5 × 10⁷ F-P815 cells or 10⁷ P815 i.p. on day -9. Helper activity was assayed as in Table 1. Experiment 2: C57BL/6J mice were immunised with 5 × 10⁷ F-P815 cells on day -9 and spleens assayed for helper activity. For anti-θ serum treatment¹³ 2 × 10⁷ spleen cells were incubated in 1 ml BSS and 0.1 ml anti-θ serum for 30 min at 0° C. Cells were washed and resuspended in 1 ml BSS containing 0.15 ml guinea pig complement⁹ and incubated 30 min at 37° C. Controls were incubated with complement only.

the H-2d antigenic determinants on P815 cells (R. Hyman and G. Dennert, unpublished results), it is possible that carrier priming with fixed P815 cells is in fact a low dose priming insufficient to prime T killer cells. If this reasoning is correct, then low doses of normal P815 cells should also prime for helper activity. Table 4 shows that this is the case. P815 cells were irradiated with 2,000 r. to inhibit multiplication and injected in various doses into animals. It is seen that helper activity is found in spleens injected with low doses (2 × 10⁶ and 4 × 10⁶ P815 cells per mouse) but not in mice injected with a higher dose (10⁷) of P815 cells. The latter dose was also insufficient to induce cytotoxic T cells in these mice and it was subsequently found that ten times higher doses of irradiated cells have to be injected to prime for T killer cells (unpublished results). These experiments therefore seem to support the idea that carrier priming by fixed P815 cells is actually due to a low dose priming. Priming with fixed P815 cells however is usually superior to priming with low doses of normal P815 cells. This was also found in experiments where carrier priming to normal and fixed erythrocytes was studied⁸.

Though it had previously been shown⁶ that cytotoxicity of

Table 4 Carrier priming by fixed and normal P815 cells

<i>In vivo</i> immunogen	10^6 TNP-P815 per culture	P.F.C. per 10^6 on TNP-BRBC
—	—	1
—	+	<1
5×10^7 F-P815	—	35
5×10^7 F-P815	+	446
10^7 P815 2,000 r.	—	3
10^7 P815 2,000 r.	+	<1
2×10^6 P815 2,000 r.	—	11
2×10^6 P815 2,000 r.	+	54
4×10^6 P815 2,000 r.	—	9
3×10^6 P815 2,000 r.	+	102

C57BL/6J mice were injected with varying doses of F-P815 and normal P815, irradiated with 2,000 r. On day 11, spleens were collected and tested for cytotoxicity and carrier priming. None of the groups showed any cytotoxic activity in their spleens.

P815 immunised C57BL/6J spleen cells is effected by thymus-derived lymphocytes, it had to be demonstrated that the helper activity observed in F-P815 primed spleen cells is also due to T cells. Treatment of F-P815 primed spleens with anti- θ serum¹³ and complement completely abrogated the helper activity of these spleens, confirming that this activity is mediated by thymus-derived lymphocytes (Table 3, experiment 2).

In summary, these experiments show that under appropriate immunisation conditions either helper T cells or killer T cells can be found in sensitised spleens: spleens which show helper activity are not cytotoxic and spleens which are cytotoxic show no helper activity. Since it is found, however, that cytotoxic spleens inhibit the helper activity of F-P815 primed spleen cells it cannot be excluded that cytotoxic spleens contain T helper cells.

The experiments do not show that low doses of irradiated P815 or F-P815 prime directly for T helper cells for the following reason: the T helper assay *in vitro* takes 5 d of incubation, while the cytotoxic assay takes only a few hours. Thus, in culture maturation of a T helper precursor to T helper could occur. These experiments, however, strongly argue that the helper cell (or the precursor thereof) and the cytotoxic killer cell are likely to be two different subsets of T cells.

Recently other subsets of T cells with specific functions have been described¹⁴. Cantor and Asofsky demonstrated the requirement of two cells with different sensitivities to anti-thymocyte serum in a graft-versus-host reaction¹⁵. Bach *et al.*¹⁶ demonstrated the existence of two populations of cells, one responding in the mixed lymphocyte reaction and the other effecting cell mediated lysis. It is therefore not surprising that T helper and T killer cells are found here to be different cell types. Further experiments must be directed toward determining the relationships between the various subsets of T cells so far described.

I thank R. Austin for assistance and Dr Edwin S. Lennox for discussion and financial support. Funds were provided by a grant from the National Institutes of Health to Edwin S. Lennox, and from The Edna McConnell Clark Foundation, The Korda Foundation, and The American Cancer Society.

GUNTHER DENNERT

The Salk Institute for Biological Studies,
and the Armand Hammer Center for Cancer Biology,
P O Box 1809,
San Diego, California 92112

Received December 27, 1973.

- ¹ Schimpl, A., and Wecker, E., *Eur. J. Immun.*, **1**, 304 (1971).
- ² Dennert, G., and Lennox, E. S., *Nature new Biol.*, **243**, 214 (1973).
- ³ Kreth, H. W., and Williamson, A. R., *Nature*, **234**, 454 (1971).
- ⁴ Dennert, G., and Lennox, E. S., *Nature new Biol.*, **238**, 114 (1972).
- ⁵ Dennert, G., and Lennox, E. S., *J. Immun.*, **111**, 1844 (1973).
- ⁶ Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *Nature*, **228**, 1308 (1970).

- ⁷ Nordin, A. A., Cerottini, J. C., and Brunner, K. T., *Eur. J. Immun.*, **1**, 55 (1971).
- ⁸ Dennert, G., and Tucker, D. F., *J. exp. Med.*, **136**, 656 (1972).
- ⁹ Mishell, R. I., and Dutton, R. W., *J. exp. Med.*, **126**, 423 (1967).
- ¹⁰ Rittenberg, M. B., and Pratt, K. L., *Proc. Soc. exp. Biol. Med.*, **132**, 575 (1969).
- ¹¹ Jacobson, E. B., and Herzenberg, L. A., *J. exp. Med.*, **135**, 1151 (1971).
- ¹² Falkoff, R., and Kettman, J., *J. Immun.*, **108**, 54 (1972).
- ¹³ Reif, A. E., and Allen, J. M. V., *J. exp. Med.*, **120**, 413 (1964).
- ¹⁴ Raff, M. C., and Cantor, H., *Progr. Immun.*, **1**, 83 (1971).
- ¹⁵ Cantor, H., and Asofsky, R., *J. exp. Med.*, **135**, 764 (1972).
- ¹⁶ Bach, F. H., Segall, M., Stouber Zier, K., Sondel, P. M., Alter, B. J., and Bach, M. L., *Science*, **180**, 403 (1973).

Type-specific protein in herpes simplex virus envelope reacts with neutralising antibody

CERVICAL carcinoma has been correlated with previous infection with herpes simplex virus type 2 detected serologically¹⁻³. These tests have, however, been confused by the fact that type 1 and type 2 herpes simplex viruses cross neutralise and it is therefore difficult to use the data to provide clear evidence for previous type 2 infection⁴. We have previously shown that antisera to types 1 and 2 viruses contain antibodies to a structural antigen, Band II (ref. 5), which neutralise both types of virus⁶. By indirect absorption methods, we have shown that they also contain antibodies to type-specific proteins which neutralise the homologous type only⁶.

We now present more direct evidence for the existence of such proteins by showing that antiserum to a glycoprotein of enveloped type 1 virus particles neutralises type 1 but not type 2 virus. These studies indicate that it should be possible to prepare type-specific antigens which could be used in seroepidemiological surveys to provide conclusive evidence of previous type 2 infection.

Enveloped particles of herpes simplex virus type 1 were obtained by precipitation by polyethylene glycol of the virus released into the supernatant growth medium of HEP 2 cells infected with herpes simplex type 1 (strain HFEM). The virus was purified by two cycles of sedimentation in sucrose gradients. Figure 1 shows the pattern of polypeptides revealed by electrophoresis in SDS polyacrylamide gels of the purified virus after disruption with SDS and dithiothreitol. The region marked VP2/3 has been shown to correspond to glycosylated polypeptides not present in naked virus particles (K.L.P., and D.H.W., unpublished). A longitudinal slice was removed

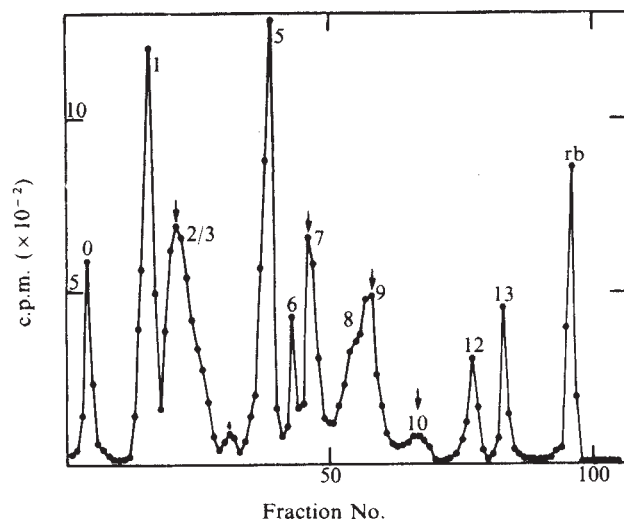


Fig. 1 Electrophoresis of disrupted enveloped Herpes simplex virus particles on SDS-polyacrylamide gel. Direction of migration from left to right (that is, higher molecular weight polypeptides to the left). Peaks identified as glycoproteins by glucosamine labelling are arrowed. Peak 1 is the major capsid polypeptide corresponding to VP5 of Spear and Roizman⁸ and peak 2/3 (VP2/3) most likely corresponds to their broad glycoprotein peak VP7/8.

from other gels to identify the VP2/3 region and the appropriate segments removed from the remaining portion of the gels. The segments were macerated and injected with Freund's incomplete adjuvant into the footpads of rabbits. From the intensity of stain in the gel slices we estimate that about 40 µg protein was inoculated. A second injection was given 2 weeks later and the rabbits bled after a further 10 d.

The resulting antiserum was tested in neutralisation tests against herpes simplex type 1 strain HFEM and herpes simplex type 2 strain 3345 as previously described⁶. The neutralisation rate constant against type 1 virus was 0.167 (mean of seven determinations) and against type 2 virus was 0.001 (mean of nine determinations) which did not differ significantly from zero. In contrast, antiserum to Band II had neutralisation rate constants against type 1 virus of 0.18 and against type 2 virus of 0.06 (ref. 6). The neutralising activity of the antiserum to VP2/3 was unaffected by absorption with an extract of cells infected with herpes simplex virus type 2, but was completely abolished by absorption with an extract of cells infected with herpes simplex virus type 1. The neutralisation rate constant of antiserum to Band II antigen, however, was reduced by 90% after absorption with the same extract of cells infected with type 2 virus (Table 1).

Table 1 Proof of type specificity of antiserum to VP2/3 polypeptide by absorption tests.

Antiserum	Absorbed with	Neutralisation rate constant of absorbed serum for type 1 virus
Anti VP2/3	Extract of uninfected cells	0.120
Anti VP2/3	Extract of cells infected with type 1 virus	0
Anti VP2/3	Extract of cells infected with type 2 virus	0.132
Anti Band II	Extract of uninfected cells	0.644
Anti Band II	Extract of cells infected with type 1 virus	0
Anti Band II	Extract of cells infected with type 2 virus	0.052

The antiserum to VP2/3 had similar neutralisation rate constants in tests against two other type 1 strains but gave no significant neutralisation of two other type 2 strains. The type specificity was confirmed by complement fixation tests (Table 2).

Table 2 Type specificity of antiserum to VP2/3 polypeptide demonstrated by complement fixation

Antiserum	Complement fixing titre* with Type 1 antigen	Type 2 antigen
Anti-VP2/3	1280	<40†
Anti-type 1 virus	2560	5120
Anti-type 2 virus	2560	2560

* Reciprocal of highest dilution fixing 1.5 units of complement using optimum dilutions of antigens.

† Antiserum had anticomplementary activity at lower dilutions.

An antiserum with similar neutralising properties was obtained by an alternative method. Antiserum to type 1 virus, rendered selective for type-specific antigens by absorption with an extract of type 2 virus-infected cells⁶, was used to detect type-specific antigens in fractions obtained by preparative polyacrylamide gel electrophoresis (without SDS) of an infected cell extract⁷. Precipitin bands obtained in agar gel immunodiffusion of one type specific fraction and the antiserum, were injected into the footpad of rabbits. This antiserum and antiserum to VP2/3 gave single precipitin bands in agar gel immunodiffusion tests with extracts of cells infected with type 1 virus. The precipitin bands obtained with the two antisera showed reaction of identity. Neither antisera gave any reaction in immunodiffusion tests with extracts of cells infected with type 2 virus.

The results provide direct evidence for the presence of a type-specific antigen both in infected cells and as a glyco-

protein on the surface of enveloped particles, whose interaction with its corresponding antibody leads to a loss of virus infectivity. They offer some hope that it may be possible to prepare a type-specific reagent for detection of previous type 2 infection in seroepidemiological surveys.

We thank the Medical Research Council for a research grant, and the Science Research Council for a research studentship (K.L.P.).

Department of Virology,
Medical School,
Birmingham, B15 2TJ

Department of Microbiology,
School of Medicine,
Leeds LS2 9NL

Received January 14, 1974.

Note added in proof: Since this work was completed we have heard that Dr R. J. Courtney (Baylor University College of Medicine, Houston, Texas) has prepared an antiserum with similar properties using extracts of cells infected with herpes simplex virus type 1. This antiserum also had type specific neutralising activity.

*Present address: Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas 77025.

¹ Rawls, W. E., Laurel, D., Melnick, J. L., Glicksman, J. M., and Kaufman, R. H., *Am. J. Epidemiol.*, **87**, 647 (1968).

² Nahmias, A. J., Josey, W. W., Naib, Z.M., Luce, C. F., and Duffey, A., *Am. J. Epidemiol.*, **91**, 539 (1970).

³ Skinner, G. R. B., Thoulless, M. E., and Jordan, J. A., *J. Obst. Gynec.*, **78**, 1031 (1971).

⁴ Wildy, P., *Cancer Res.*, **33**, 1465 (1973).

⁵ Watson, D. H., and Wildy, P., *J. gen. Virol.*, **4**, 163 (1969).

⁶ Sim, C., and Watson, D. H., *J. gen. Virol.*, **19**, 217 (1973).

⁷ Watson, D. H., *J. gen. Virol.*, **4**, 151 (1969).

⁸ Spear, P., and Roizman, B., *J. Virol.*, **9**, 143 (1972).

***In vitro* generation of B lymphocytes in mouse foetal liver, a mammalian 'bursa equivalent'**

THERE is good evidence that in birds a haemopoietic stem cells of yolk sac origin^{1,2} differentiate into immunoglobulin-bearing lymphocytes in the bursa of Fabricius³. The differentiated lymphocytes then migrate to the peripheral lymphoid tissues^{4,5}, where they constitute the bursa-derived B lymphocyte population. The primary site(s) of B lymphocyte development in mammals, however, has remained a mystery, with gastrointestinal associated lymphoid tissues⁶ (including appendix⁷, tonsils⁸ and Peyer's patches⁹) and haemopoietic tissues¹⁰⁻¹² generally considered the main candidates for mammalian 'bursa equivalent'. Here we report the development of IgM, IgG and probably IgA-bearing lymphocytes in organ cultures of mouse foetal liver removed at 14 d gestation, a time well before immunoglobulin-bearing cells or cells of lymphoid morphology could be detected in the intact animal. These studies indicate that foetal liver is a bursa equivalent in mice and that gastrointestinal lymphoid tissues are not a *sine qua non* for the initial development of B cells. In addition, they provide evidence that IgG and probably IgA-bearing B lymphocytes can develop in the absence of T lymphocytes.

Foetal livers were removed from BALB/c embryos of 14 or 15 d gestation (the day of appearance of a vaginal plug being taken as day 0) and chopped with fine knives. Small explants (2-4 mm diameter) were placed on ultrathin Millipore filters (type TH), floating on 1 ml of RPMI 1640 medium containing 10% heat-inactivated foetal calf serum in the centre well of Falcon organ culture dishes (catalogue No. 3010), the outer trough being filled with distilled water. The dishes were incubated at 37°C in an atmosphere of 5% CO₂ in air. Foetal livers, spleens or cultured explants were

Table 1 Properties of fluorescein-conjugated antibodies

Antiserum	Abbreviation	Fraction used	F/P Ratio	Concentration used (mgml ⁻¹)	References	Proportion of cells labelled in adult spleen (%)
Rabbit anti-mouse immunoglobulin	Anti-MIg-fl	Purified antibody	1.5	2.0	14, 25	52.6*
Rabbit anti-mouse immunoglobulin	Fab Anti-MIg-fl	Fab IgG	2.1	10.0	25	40-55
Goat anti-mouse μ (IgM)	Anti- μ -fl	Purified antibody	3.0	0.5	21	30.6*
Goat anti-mouse γ_1 (IgG ₁)	Anti- γ_1 -fl	" "	3.2	0.5	21	0.3*
Goat anti-mouse γ_{2ab} (IgG _{2a} and IgG _{2b})	Anti- γ_{2ab} -fl	" "	3.5	0.5	21	18.8*
Goat anti-mouse α (IgA)	Anti- α -fl	" "	3.2	0.5	21	0.7*
Goat anti-human μ (IgM)	Anti-human μ -fl	" "	3.2	0.5	16	0
Mouse anti- θ C3H	Anti- θ -fl	IgG	0.8	10.0	15	25-40

The aggregate value for labelling with these reagents separately is 50.4%.

*Result of a single experiment where the proportion of cells stained with a mixture of fluorescein-fl-labelled anti- μ , anti- γ_1 , anti- γ_{2ab} and anti- α was 47%.

Table 2 Proportion of Ig-bearing and θ -bearing cells in foetal liver and spleen*

Day of gestation	No. of experiments	Liver						Spleen					
		Ig.	μ	γ_1	γ_2	α	θ	Ig	μ	γ_1	γ_2	α	θ
12	1	$0/10^4$	—	—	—	—	$0/10^4$	0	—	—	—	—	—
13	1	$0/1.2 \times 10^4$	—	—	—	—	$0/2 \times 10^4$	0	—	—	—	—	—
14	7	$0/1.6 \times 10^5$	—	—	—	—	$0/2.7 \times 10^4$	2	$0/600$	—	—	—	—
15	1	$0/10^4$	—	—	—	—	—	0	—	—	—	—	—
16	2	$0/10^4$	$0/6 \times 10^4$	$0/5 \times 10^4$	$0/5 \times 10^4$	$0/5 \times 10^4$	$0/10^4$	2	$0/5500$	—	$0/200$	—	—
17	1	$11/10^3$ (1.1%)	—	—	$3/1.5 \times 10^4$ (0.02%)	$0/10^4$	$0/3 \times 10^3$	1	$7/588$ (1.3%)	$7/504$ (1.2%)	$2/5000$ (0.04%)	$0/12000$	—
18	2	$5/10^3$ (0.5%)	$35/6500$ (0.5%)	$0/10^5$	$5/10^4$ (0.05%)	$0/10^4$	$2/2 \times 10^4$ (0.01%)	2	$6/500$ (1.2%)	$13/313$ (4.2%)	$7/2000$ (0.35%)	$2/7500$ (0.03%)	$5/2600$ (0.2%)
19	1	$23/2400$ (1%)	$41/3000$ (1.4%)	$0/5000$	$5/2500$ (0.2%)	$0/5000$	—	1	$13/401$ (3.9%)	$3/306$ (2%)	$0/5000$	$3/1250$ (0.24%)	$0/4000$
20	0	—	—	—	—	—	—	2	—	$16/500$ (3.2%)	$0/2000$	?	—

*Slides were scanned systematically using an oil-immersion objective and the total number of cells surveyed was estimated from the number of fields and number of cells per field examined.

Table 3 Development of Ig-bearing lymphocytes in foetal liver explant*

Experiment	Age of liver explanted (d)	Days in culture	Proportion of cells labelled with							
			Anti-MIg	Fab anti-MIg	Anti- μ	Anti- γ_1	Anti- γ_2	Anti- α	Anti- θ	Anti-human μ
1	14	2	$0/7000$	—	—	—	—	—	$0/2000$	—
2	14	4	$0/2000$ (0.45%)	—	—	—	—	—	$0/4000$	—
3	14	4	—	$5/2500$ (0.2%)	$7/3000$ (0.23%)	$0/8000$	$3/7300$ (0.04%)	$0/4000$	$0/8000$	—
4	15	3	$2/2000$ (0.1%)	$2/2000$ (0.1%)	—	—	—	—	—	—
5	14	5	—	$23/1900$ (1.2%)	$18/1280$ (1.4%)	—	$5/3100$ (0.16%)	—	—	—
6	14	5	—	—	$23/2500$ (0.92%)	$0/3500$	$3/3000$ (0.1%)	$0/4000$	$0/2000$	$0/6000$
7	14	7	—	$12/440$ (2.5%)	$7/232$ (3%)	$0/200$	$3/200$ (1.5%)	$2/4000$ (0.05%)	—	$0/5000$

*See legend to Table 2.

taken at various times and teased in veronal-buffered saline containing 20% foetal calf serum and 0.2% sodium azide. Aliquots of cells were spun onto slides in a cytocentrifuge and stained with McNeal's tetrachrome. In addition, cells were incubated in suspension with a variety of fluorescein-conjugated antisera (see Table 1) at 0°C for 30 min, washed twice and examined for surface fluorescence using a modified Vicker's M40 microscope equipped for incident illumination as previously described¹³. Fluorescein-conjugated anti-immunoglobulin antibodies, polyspecific and class specific, were used to detect B lymphocytes¹⁴ while a fluorescein-conjugated anti- θ serum was used to detect T cells¹⁵. All of these reagents have been used in previously reported experiments and their properties are outlined and referenced in Table 1. Usually the antibodies used were purified and absorbed using solid-phase immunoabsorbents. The proportion of cells labelled with the various reagents in adult BALB/c spleens is indicated in Table 1.

Differential cell counts on cytocentrifuge preparations from freshly teased (uncultured) tissues showed that most of the cells prepared from 12-19 d foetal liver belonged to the erythroid cell series. From 16 d, increasing numbers of granulocytic and monocytic cells were seen with the first lymphoid cells being detected at 17 d. We could distinguish lymphocytes from erythroid cells because lymphocytes have a thinner rim of cytoplasm which is paler staining than that of the erythroid series of cells. The picture in foetal spleen was similar to that in liver except that a greater proportion of granulocytic and monocytic cells were present at all stages. By fluorescence, immunoglobulin-bearing cells were first detected at 17 d gestation in both liver and spleen (Table 2) correlating well with the morphological studies. As has been shown in man¹⁶, IgM and IgG-bearing cells developed before IgA-bearing lymphocytes. It seems that B cell development is relatively retarded in BALB/c mice compared to other strains where B cells have been detected

at 16 d gestation^{11,17}. In agreement with this we have found that CBA mice have a higher proportion of B cells in their spleens at birth than do BALB/c mice.

Results of 14 d foetal liver organ culture experiments are outlined in Table 3. Although no cells of lymphocyte morphology were found in 14 d livers, after 4 d in culture approximately 0.5% of the nucleated cells were typical small lymphocytes on morphological criteria. With time in culture, erythropoiesis declined and an increasing percentage of the cells present were granulocytic, monocytic and lymphoid, with approximately 2–3% of the cells being lymphocytes after 7 d in culture. Correlating well with the morphological studies, 0.2–0.45% of cells from explants in culture for 4 d bore immunoglobulin, mostly IgM, with a smaller proportion of IgG₂-bearing cells. The proportion increased with time in culture, so that at 7 d between 2–3% of the cells were immunoglobulin-positive, and two cells (out of 4000 scanned) were found that stained distinctly, but faintly, with anti- α -fluorescein(f). No cells labelled with anti- θ -f, anti- γ -f or anti-human- μ -f after 4 or 5 d in culture, while an appropriate proportion labelled with Fab anti-MIg-f, excluding the possibility that fluorescent labelling was related to the binding of the conjugates by Fc receptors of cells and indicating that θ -bearing T cells did not develop in these cultures.

It is highly unlikely that the immunoglobulin-bearing cells detected after 4 d in culture were present in the 14 d liver explants at the beginning of the culture period and then merely concentrated by selective cell survival, as no immunoglobulin-bearing cells were detected in scanning more than 0.4×10^6 cells from 14–16 d livers, including samples from all livers that were subsequently cultured. Although there was a selective loss of erythroid cells during culture, we were able to collect 3×10^6 – 6×10^6 cells following 4–7 d culture of 2 or 3 14 d livers (about 3×10^7 can be collected by teasing from this number of livers). Even with this cell loss, if the B cells which we detected after culture (up to 3% of the cells collected) were already present before culture, we would have detected them among the large number of 14 d foetal liver cells examined.

These experiments show that immunoglobulin-bearing B lymphocytes can differentiate within mammalian foetal liver, *in vitro*. On this basis mammalian foetal liver can be considered as a possible candidate for a mammalian 'bursal equivalent'. We believe that the liver provides a 'micro-environment' for B cell differentiation as we failed to find immunoglobulin-bearing cells in 5 d cultures of liver cells obtained from 14 day foetal liver either by trypsinisation or by gently teasing the tissue apart. The cells were cultured in Falcon flasks in exactly the same media and atmosphere as adopted for the whole organ culture. The possibility that B cells can also develop in other tissues, such as the appendix and Peyer's patches, has not been excluded, although the intestinal epithelial theory of B cell maturation^{6,18,19} is no longer tenable in its original form. It will be of interest to investigate the capacity of other tissues, such as foetal spleen and bone marrow, to generate B cells *in vitro*, to determine if all haemopoietic tissues can serve this function, or whether foetal liver, which is largely derived from foregut epithelium, is the only bursa-equivalent organ. These experiments also demonstrate that IgM, IgG and probably IgA-bearing cells can develop in the foetal liver, in the absence of T cells, providing the most direct evidence thus far that T cells are not required for the postulated switch from IgM to IgG and/or to IgA in single B cells^{20–23}. The failure of θ -bearing T lymphocytes to develop in 5d cultures is of interest in view of the recent evidence that agents such as poly(A)-poly (U), cyclic AMP and lipopolysaccharide can induce some cells in 14 d foetal liver to express θ (ref. 24). Our attempts, in collaboration with Peter Beverley, to induce the appearance of immunoglobulin-or θ -bearing cells during a 2 h incubation of 14 d BALB/c foetal liver cells with these

agents have failed so far.

The development of B lymphocytes in 14 d foetal liver explants should provide a useful system for investigating the microenvironmental requirements for B cell differentiation, the putative immunoglobulin class switch, tolerance induction, and the generation of antibody diversity in the absence of T cells and in a relatively controlled antigenic environment. Elucidation of the bursa equivalent is of obvious importance to the understanding of a variety of immune deficiency diseases.

JOHN J. T. OWEN*
MAX D. COOPER†
MARTIN C. RAFF

Imperial Cancer Research Fund,
Tumor Immunology Unit and the MRC Neurology Project,
Zoology Department,
University College London,
London WC1 6BT

Received January 21; revised February 22, 1974.

*Present address: Department of Anatomy, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne 1.
†On leave from Department of Pediatrics and Microbiology, University of Alabama in Birmingham, U.S.A.

- ¹ Moore, M. A. S., and Owen, J. J. T., *Devl Biol.*, **14**, 40 (1966).
- ² Moore, M. A. S., and Owen, J. J. T., *Nature*, **215**, 1081 (1967).
- ³ Kincade, P. W., and Cooper, M. D., *J. Immun.*, **106**, 1421 (1971).
- ⁴ Cooper, M. D., Schwartz, M. M., and Good, R. A., *Science*, **151**, 471 (1966).
- ⁵ Durkin, H. G., Theis, G. A., and Thorbecke, G. J., *Morphological and Functional Aspects of Immunity* (edit. by Lindahl-Kiessling, K., Alm, G., and Hanna, M. G.) 119 (Plenum Press, New York, 1971).
- ⁶ Cooper, M. D., Perey, D. Y., McKneally, M. F., Gabrielson, A. E., Sutherland, D. E. R., and Good, R. A., *Lancet*, **i**, 1388 (1966).
- ⁷ Archer, O. K., Sutherland, D. E. R., and Good, R. A., *Nature*, **200**, 337 (1963).
- ⁸ Peterson, R. D., Cooper, M. D., and Good, R. A., *Am. J. Med.*, **38**, 579 (1965).
- ⁹ Perey, D. Y. E., Cooper, M. D., and Good, R. A., *Science*, **161**, 265 (1968).
- ¹⁰ Ibrahim, F., and Osmond, O. G., *Anat. Rec.*, **168**, 139 (1970).
- ¹¹ Nossal, G. J. V., and Pike, B. L., *Immunology*, **25**, 33 (1973).
- ¹² Abdou, N. I., and Richter, M., *Adv. Immun.*, **11**, 202 (1969).
- ¹³ Ashman, R. F., and Raff, M. C., *J. exp. Med.*, **137**, 69 (1973).
- ¹⁴ Raff, M. C., *Immunology*, **19**, 637 (1970).
- ¹⁵ Raff, M. C., *Nature new Biol.*, **246**, 350 (1973).
- ¹⁶ Lawton, A. R., Self, K. S., Royal, S. A., and Cooper, M. D., *Clin. Immunobiol., Immunopath.*, **1**, 104 (1972).
- ¹⁷ Spear, P. G., Wang, A., Rutishauser, U., and Eidelman, G. M., *J. exp. Med.*, **138**, 557 (1973).
- ¹⁸ Fichtelius, K. E., *Exp. Cell. Res.*, **49**, 87 (1968).
- ¹⁹ Cooper, M. D., and Lawton, A. R., *Contemporary Topics in Immunobiology*, (edit. by Hanna, M. G.), **49**, (Plenum Press, New York, 1972).
- ²⁰ Kincade, P. W., Lawton, A. R., Bockman, D. E., and Cooper, M. D., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1918 (1970).
- ²¹ Lawton, A. R., Asofsky, R., Hylton, M. B., and Cooper, M. D., *J. exp. Med.*, **135**, 277 (1972).
- ²² Pernis, B., Forni, L., and Amante, L., *Ann. N.Y. Acad. Sci.*, **190**, 420 (1971).
- ²³ Pink, R., Wang, A. C., and Fudenberg, H. H., *A. Rev. Med.*, **22**, 145 (1971).
- ²⁴ Scheid, M. P., Hoffmann, M. K., Komur, K., Hammerling, U., Abbott, J., Boyse, E. A., Cohen, G. H., Hooper, J. A., Schulof, R. S., and Goldstein, A. L., *J. exp. Med.*, **138**, 1027 (1973).
- ²⁵ Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225 (1971).

Expression of M locus differences by B cells but not T cells

Within or close to the major histocompatibility complex (MHC) of man and mouse there are genetic loci which control the production of lymphocyte-associated determinants capable of stimulating allogeneic lymphocytes to synthesise DNA in

Table 1 MLR between cells from MHC and M different mice

Responder	Stimulator					
	CBA/J spleen	CBA/J thymus	C3H/HeJ spleen	BALB/cJ spleen	DBA/2J spleen	DBA/2J thymus
CBA/J thymus	4,167 $\pm$ 503	—	4,251 $\pm$ 306	19,194 $\pm$ 2701	—	—
C3H/HeJ thymus	32,979 $\pm$ 1,269	2,143 $\pm$ 160	2,140 $\pm$ 310	—	—	—
BALB/cJ	—	13,802 $\pm$ 2,004	—	3,131 $\pm$ 460	41,103 $\pm$ 3,231	2,017 $\pm$ 126
DBA/2J thymus	33,683 $\pm$ 3,975	—	—	3,204 $\pm$ 706	3,918 $\pm$ 401	—

Numbers represent c.p.m. $\pm$ s.d.

mixed lymphocyte culture^{1,2}. In the mouse the mixed lymphocyte reaction (MLR) is also controlled by an additional locus, the M locus, which is not linked to MHC³. These MLR loci seem to be distinct from the loci coding for serologically defined (SD) antigens^{1,2,4}. Recent studies suggest that, in certain situations, a genetic difference between responder and stimulator cells is not an absolute requirement in MLR. Thus, Howe *et al.*⁵ and Boehmer *et al.*^{6,7} found that thymus cells from certain mouse strains produce high levels of thymidine incorporation when cultured with syngeneic B lymphocytes.

Lymphocytes which proliferate in response to cells differing at lymphocyte defined (LD) loci, but not at SD loci, are reported to be unable to develop into cytotoxic effector cells^{1,2,4}. The nature of the LD stimuli which induce cell proliferation in this situation is unknown. This paper attempts to define the cells which carry these determinants.

We have employed Hannig's system of preparative cell electrophoresis⁸ to prepare highly purified T and B cell populations for use as stimulating cells in MLR. BALB/c (H2^d, M1s^b), DBA/2 (H2^d, M1s^b), C3H (H2^k, M1s^a) and CBA/J (H2^k, M1s^a) mice were used in these studies. BALB/c and DBA/2 are MHC identical but differ at the M locus; a similar relationship exists between C3H and CBA/J.

Thymus cells, thoracic duct lymphocytes (TDL) and spleen cells were obtained by standard methods⁹. TDL were used as stimulating cells after separation by electrophoresis as described previously¹⁰. Thymocytes from 6-week-old mice served as responder cells. These cells were cultured with stimulator cells treated with mitomycin C¹¹ from thymus, spleen or TDL in Falcon plastic tubes 2005 (Gateway International, Los Angeles) at a total cell concentration of 5×10^6 per ml culture medium. With thymus cells as stimulators, the ratio of responding to stimulating cells was 3 : 1; with cells from spleen and TDL, a responder : stimulator ratio of 10 : 1 was chosen; in previous studies these ratios gave optimal stimulation with strain combinations differing at the MHC or M locus and reduced 'backstimulation' (see below) by the mitomycin C-treated cells (ref. 12 and unpublished observations). The culture medium

used was RPMI 1640 supplemented with 10% foetal calf serum and mercaptoethanol¹³. The cells were cultured at 37° C in a humidified atmosphere of 10% CO₂ in air. ³H-thymidine (Radiochemical Centre, Amersham, specific activity 25 Cimmol⁻¹) was added to the cultures at a final concentration of 1.3 μ Ciml⁻¹ after 48 h and cells were collected after 60 h. Radioactivity in DNA extracted from the cells was measured as described previously¹¹. Background thymidine incorporation was calculated from separate incubations of either 5×10^6 responder or stimulator cells.

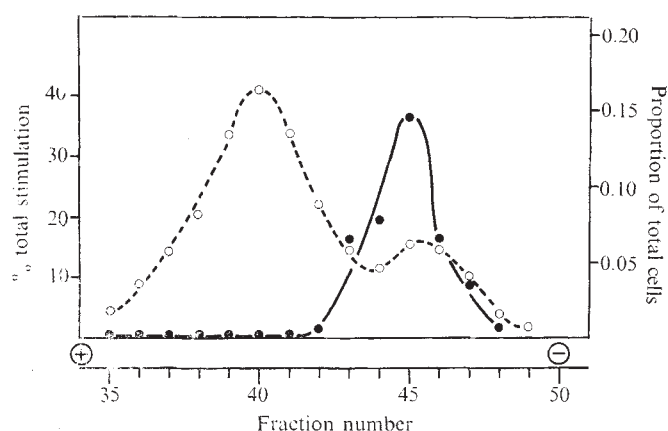


Fig. 2 Stimulating capacity of electrophoretically separated CBA TDL treated with mitomycin C cultured with C3H (M different) thymus cells. Calculated background: 2,528 c.p.m. Peak stimulation with 5×10^6 stimulators: $42,679 \pm 3,926$ c.p.m. ●—●, Stimulation; ○--○, all cells.

Table 1 shows DNA synthesis by thymus cells cultured with stimulating cells from various sources. It is apparent that there is unidirectional stimulation of BALB/c thymus by DBA/2 spleen and of C3H thymus by CBA spleen. These findings are in agreement with the studies of Festenstein¹⁴ and Salaman *et al.*¹⁵. They differ to some extent from the findings of Katz-Heber *et al.*¹⁶. These workers found that while BALB/c cells responded strongly to DBA/2, a significant though weak response was also observed in the reverse direction. The authors used a low ratio of responder to stimulator cells in their cultures. Recent work of one of us (H.v.B.) suggests that, under these conditions, the response observed by Katz-Heber *et al.* when BALB/c cells were used as the stimulating population might have reflected 'backstimulation' of the DBA/2 cells. In this respect, significant DNA synthesis was observed when parental cells treated with mitomycin C were cultured at a low (but not a high) ratio with semiallogeneic F₁ hybrid cells. From additional evidence it was concluded that DNA synthesis in this situation represented backstimulation of F₁ cells responding to a 'recognition signal' from the T cells in the parental population treated with mitomycin C^{17,18}.

When thymus cells instead of spleen cells were used as stimulators, responses by thymus cells were significant when an MHC difference was involved but not significant when the cells differed at the M locus only (Table 1). The data in Table 1 suggest, therefore, that the lymphocyte-defined differences coded for by the M locus were carried by spleen cells but not

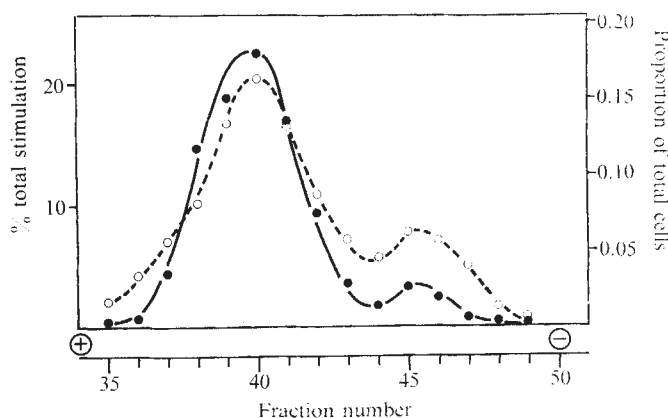


Fig. 1 Stimulating capacity of electrophoretically separated CBA TDL treated with mitomycin C cultured with BALB/c (MHC plus M different) thymus cells. Calculated background: 1,371 c.p.m. Peak stimulation with 5×10^6 stimulators: $103,428 \pm 5,630$ c.p.m. ●—●, Stimulation; ○--○, all cells.

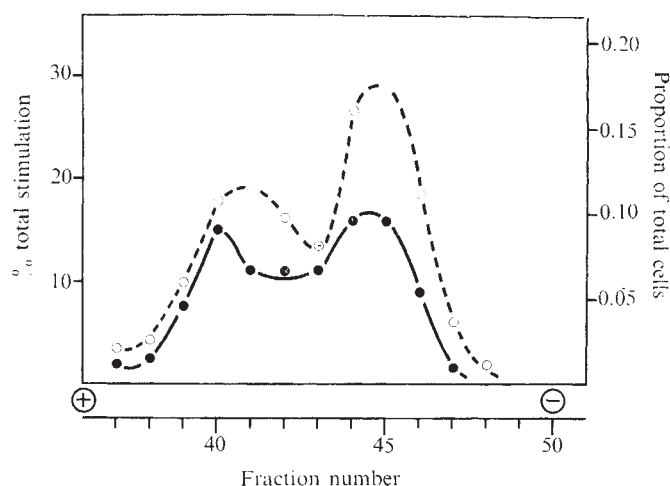


Fig. 3 Stimulation capacity of electrophoretically separated DBA/2 TDL treated with mitomycin C cultured with CBA/J (MHC plus M different) thymus cells. Calculated background: 3,049 c.p.m. Peak stimulation with 5×10^6 stimulators: $41,683 \pm 1,050$ c.p.m. (DBA/2 TDL were obtained 48 h after cannulation and have therefore a higher proportion of B lymphocytes.) ●—●, Stimulation; ○—○, all cells.

by thymus cells. More precise information on this point was obtained by testing the stimulating capacity of purified populations of mature (small) T and B lymphocytes obtained from thoracic duct lymph.

TDL subjected to electrophoresis separate into two distinct cell populations which partly overlap¹⁹. Previous studies showed that T (θ positive) and B (immunoglobulin positive) lymphocytes were arranged in Gaussian distribution in the fast moving and slow moving cell fractions, respectively. Thus the fractions of highest electrophoretic mobility consisted of pure T cells and the fractions of lowest electrophoretic mobility of pure B cells; intermediate fractions contained both T and B lymphocytes.

Electrophoretically separated cell fractions of TDL from CBA mice were treated with mitomycin C and cultured separately at a ratio of 1:10 with thymus cells differing at the M locus (C3H) or at both MHC and M locus (BALB/c). The stimulation observed in these cultures is shown in Figs 1 and 2. As described elsewhere¹⁸, the data are expressed as the proportional stimulation provided by each stimulator fraction. To make this calculation, the counts above background given by the aliquot (0.5×10^6 cells) of each fraction of stimulating cells was

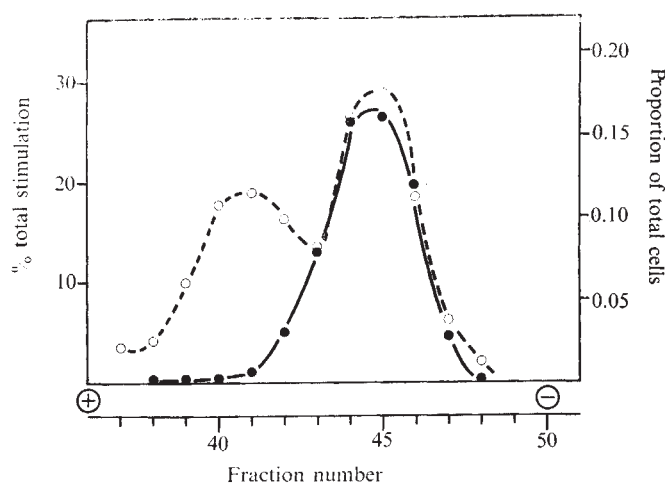


Fig. 4 Stimulation capacity of electrophoretically separated DBA/2 TDL treated with mitomycin C cultured with BALB/c (M different) thymus cells. Calculated background: 1,537 c.p.m. Peak stimulation with 5×10^6 stimulators: $66,003 \pm 9,803$ c.p.m. ●—●, Stimulation; ○—○, all cells.

multiplied by the total number of cells in that fraction. Under the constant culture conditions used, addition of the values obtained for each cell fraction provided an estimate of the total stimulating capacity of the TDL populations as a whole. The proportional stimulation provided by each fraction was then calculated with reference to this combined value.

When the data are expressed in this fashion, it is evident that, with MHC differences, thymus cells responded to TDL fractions containing either T cells, B cells or mixtures of T and B cells to approximately the same extent. With a difference restricted to the M locus, by contrast, thymus cells responded only to cells from fractions containing B cells. Similarly, fractions B cell-depleted of DBA/2 TDL stimulated CBA (MHC plus M different) thymus but not BALB/c (M different) thymus (Figs 3 and 4).

The observation that T and B lymphocytes share the capacity to induce allogeneic (MHC different) T cells to synthesise DNA corroborates our previous findings^{17,20}. A consensus of opinion has yet to be obtained on this point, however, since some workers have claimed that the allogeneic stimulus is carried predominantly by B cells¹⁹, though others maintained it was only found on T cells²¹. The failure of these workers to use more precisely defined populations of T and B cells in their studies might have contributed to the disparity in their results.

We do not know the significance of the finding that, in the MLR, the stimulus to lymphocytes differing solely at the M locus is carried exclusively by B lymphocytes. As mentioned previously, B lymphocytes activated solely by M locus differences were reported to be unable to induce cell-mediated lympholysis (CML) (ref. 4). Phytohaemagglutinin (PHA)-transformed blasts were used as target cells for CML in this study. Our work questions the validity of this choice of target cells. Thus, in mice, PHA blasts are considered to be derived from T cells that is from cells which in our studies did not express detectable M locus differences. For this reason work is currently in progress in our laboratory to determine whether lymphocytes activated to LD differences can lyse B blast cells.

We thank Nancy Erhardt for technical help.

H. VON BOEHMER
J. SPRENT

Basel Institute for Immunology,
Grenzacherstrasse 487,
CH-4058 Basel, Switzerland

Received December 3, 1973; revised March 21, 1974.

- ¹ Eijssvoogel, V. P., et al. in *Histocompatibility Testing* (edit. by Dausset, J., and Colombani, J.), 501 (Munksgaard, Copenhagen, 1972).
- ² Bach, F. H., Widmer, M. B., Bach, M. L., and Klein, J., *J. exp. Med.*, **136**, 1430 (1972).
- ³ Festenstein, H., *Ann. N.Y. Acad. Sci.*, **921**, 567 (1966).
- ⁴ Abbasi, K., and Festenstein, H., *Eur. J. Immun.*, **3**, 430 (1973).
- ⁵ Howe, M. L., *J. Immun.*, **110**, 1040 (1973).
- ⁶ von Boehmer, H., and Byrd, W., *Nature new Biol.*, **235**, 50 (1972).
- ⁷ von Boehmer, H., *Eur. J. Immun.*, **4**, 105, (1974).
- ⁸ Hanning, K. *Free Flow Electrophoresis Handbook* (edit. by Norris, J. R., and Ribbons, D. W.), 513, (Academic, London, 1971).
- ⁹ Sprent, J., *Cell. Immun.*, **7**, 10 (1973).
- ¹⁰ von Boehmer, H., Shortman, K., and Nossal, G. J. V., *Cell. Physiol.* (in the press).
- ¹¹ Byrd, W., von Boehmer, H., and Rouse, B. T., *Cell. Immun.*, **6**, 12 (1973).
- ¹² von Boehmer, H., Shortman, K., and Adams, P. B., *J. exp. Med.*, **136**, 1648 (1972).
- ¹³ Nabholz, M., Vives, J., Young, H. M., Meo, T., Miggiano, V., Rijnbeck, A., and Shreffler, D. C., *Eur. J. Immun.* (in the press).
- ¹⁴ Festenstein, H., Abbasi, K., Sachs, J. B., and Oliver, R. T. D., *Transplant Proc.*, **4**, 219 (1972).
- ¹⁵ Salaman, M. H., Wedderburn, N., Festenstein, H., and Huber, B., *Transplantation*, **16**, 29 (1973).
- ¹⁶ Katz-Heber, E., Peck, A. B. and Click, R. E., *Eur. J. Immun.*, **3**, 379 (1973).
- ¹⁷ von Boehmer, H., in *Proc. eighth Leucocyte Culture Conference, Uppsala* (edit. by Fischer-Lindahl, K.) (1973).
- ¹⁸ von Boehmer, H., *J. Immun.*, **112**, 70 (1974).
- ¹⁹ Plate, J. M. D., and McKenzie, I. F. C., *Nature new Biol.*, **245**, 247 (1973).
- ²⁰ Sprent, J., and Miller, J. F. A. P., *Cell. Immun.*, **3**, 385 (1972).
- ²¹ MacLauren, B. P., *Clin. exp. Immun.*, **10**, 649 (1972).

Erythropoiesis in an avian thymus

As part of an ecological study of the red billed quelea, *Quelea quelea*, undertaken in Tanzania and Kenya between 1969 and 1972, the size and colour of the thymus glands in over 5,000 birds were recorded and subsequently correlated with the physiological state of the birds, such as the state and degree of moult and breeding condition. More than 200 lobes from these thymus glands were excised and examined by routine histological methods using impression smears, paraffin sections and araldite embedded sections prepared for light and electron microscopy. This work, carried out in collaboration with other workers, will be reported in full elsewhere and is briefly summarised here.

In *Quelea quelea*, as in other birds¹, the thymus consists of a number of lobes well separated from one another in two long chains one on each side of the neck. In embryos about to hatch each lobe was found to be small (less than 1 mm long) and white; but by the age of 10 d, 17 out of 60 individuals examined (28%) had pink, enlarged (over 4 mm long) thymi; and on becoming independent at 20-d-old 264 out of 275 (96%) had enlarged thymic lobes. At the commencement of the postjuvenile moult at about 9 weeks of age, 113 out of 140 (80%) young still had enlarged thymi and over the next 3 months, during the progress of this moult, the proportion fell to 54 out of 155 (35%). The fall apparently continued during the first prenuptial moult, though by this time it was difficult to distinguish first year from older adults.

Thus in queleas, as in most other animals^{2,3}, maximum thymus size is reached before the onset of sexual maturity. Thereafter the thymus is said to slowly involute with age, but this is not so in queleas where the thymus glands of adults undergo rapid cycles of enlargement and regression and there are probably several such cycles in one year.

The most remarkable changes occur in breeding adults. Queleas form highly synchronised breeding colonies, each colony lasting approximately 6 weeks (after which the adults may remain in breeding condition and move away to found other colonies⁴). As Fig. 1 shows for a colony sampled in Tanzania, the percentage of adults with enlarged thymi (there was no difference in thymus size between the sexes) was low at the start of the colony as mating and laying were under way and most individuals had regressed thymi. Then, during the incubation period, the percentage of individuals with enlarged thymi increased to a peak of 70% soon after hatching, most of the rest having thymi of intermediate size (either enlarging or regressing). Thymus activity declined markedly in the latter half of the rearing period. Similar results have been obtained from other colonies, though with less complete data. With the change from a regressed to an enlarged condition the thymic lobes also changed from white to pink or bright red, and the thymic arteries and veins became prominent.

In addition to the cycle of thymus enlargement and regression in breeding colonies enlarged thymic lobes were found in about 10% of the 919 adults collected while in prenuptial moult and in 27% of the 298 birds in postnuptial moult, though the percentages varied from year to year. When wild females were kept caged in Africa and fed food and water *ad libitum*, however only 1 out of 35 birds killed at various times during the postnuptial moult had enlarged thymi. We conclude from these results that thymus enlargement associated with moult is influenced by factors other than the moult itself.

These observations substantiate Hohn's findings^{5,6} of thymus enlargement following the breeding season (and therefore during the postnuptial moult) in adult mallard ducks, American robins and house sparrows; and the observations of Ward and D'Cruz⁷ and Anderson⁸ in which thymus enlargement in a tropical bulbul and the pheasant respectively was associated with the postnuptial moult.

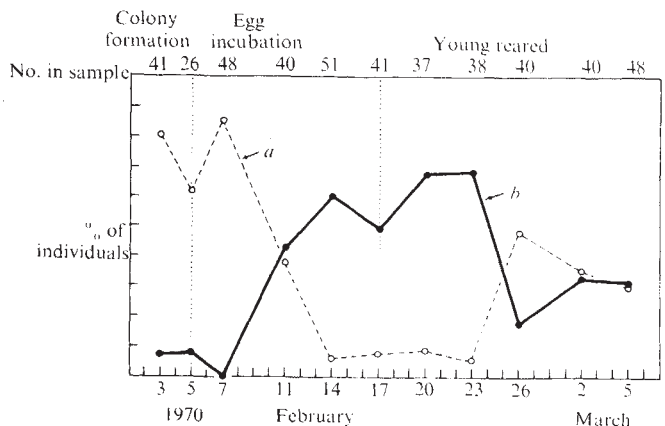


Fig. 1 Changes in the % of adult individuals with enlarged and regressed thymi in a synchronised breeding colony at Makuyuni, Tanzania. For each sample the remaining % represents birds with intermediate thymi (enlarging or regressing). The laying and hatching data are mean values for the colony. *a*, Regressed thymi; *b*, enlarged thymi.

Histologically, all except fully regressed lobes were composed of a peripheral cortex and a central medulla. The cortex of enlarging lobes contained mainly small lymphocytes (average 3–4 μ m nuclear diameter) and displayed a high level of mitosis. In the enlarged lobe the typical cortical lymphocyte was in certain regions interspersed with lymphocyte-like cells⁹, early erythroid cells (which were found by use of the analytical microscope EMMA-4 to contain iron), immature erythrocytes (polychromatophilic erythroblasts), mature erythrocytes or pyknotic cells. In the enlarged thymi of breeding adults the entire cortex of most lobes was packed with immature or mature erythrocytes with few, if any, thymic lymphocytes present; in some enlarged lobes the medulla also contained immature or mature erythrocytes. The presence of enormous numbers of erythrocytes imparted a bright red colour to the enlarged lobe which persisted in some lobes of intermediate size that we regarded as regressing. In regressing lobes, mitosis was not apparent, and the cortex contained very large numbers of erythrocytes which also filled all the blood vessels in the lobes. The medulla contained large Hassall's corpuscles which were not well developed in enlarging lobes. It seemed as though the efflux of erythrocytes from the enlarged lobes of breeding adults was sudden, for in some individuals collapsed lobes with little or no cortex were found.

Although invasion of the thymic lobes by immature erythrocytes produced elsewhere in the body cannot be entirely ruled out, the large numbers of these immature cells and the similarity between lymphocyte-like and early erythroid cells suggests that the erythrocytes were developing *in situ*. Terni¹⁰ in 1924 considered the thymus of birds to be erythropoietic; and Albert, Wolf and Pryjma¹¹ presented evidence for erythropoiesis in the mouse thymus. Sealander and Bickerstaff¹² found a rise in red cell counts correlated with increased thymus weight in wild red-backed mice. Granulopoiesis has been demonstrated in the rat thymus^{13–17} and we have found immature granulocytes (myelocytes) on electron micrographs of the *Quelea quelea* material. Pease¹⁸ showed that the myeloblast is the primitive stem cell of the granulocytic and erythrocytic lines of cell development. Thus, erythropoiesis within the thymus is not an unprecedented concept. Identification of erythroid precursor cells is beyond the scope of this study but in view of the postulated role of the small lymphocyte in bone marrow erythropoiesis⁹, the transformation of small lymphocytes under phytohaemagglutinin^{9,20} and the similarity between the small lymphocytes of the marrow and thymus

in mice²¹ the role of the small lymphocyte in the thymus needs investigating.

The significance of thymic erythropoiesis in the life of *Quelea quelea* is still a subject for speculation. The thymus enlarges while the young bird is growing rapidly and acquiring its first (juvenile) plumage and remains enlarged in most individuals well into the postjuvenile moult. In adults, it frequently becomes enlarged during moult and, in most if not all individuals, during the middle period of a breeding session. As an individual probably rears several broods in the course of a single breeding season, and moults both before and after the breeding season, we are left with the impression that a typical quelea may experience several complete cycles of thymus enlargement and recession each year of its adult life. There is reason to believe that the thymus enlarges whenever the need for extra erythrocytes is great, for example, during initial body growth and subsequently during each moult when large numbers of new feathers are growing and during the incubation period in a breeding colony when the adults are then recovering from a period of severe adverse nutritional balance that occurs after the start of each breeding session (ref. 22 and P.W., unpublished). Inanition (in this case manifested by a great fall in the bird's fat and protein reserves) is known to inhibit erythropoiesis^{23,24}, a massive production of erythrocytes following this period (that is, during the incubation period) seems quite feasible. At all these times the 14–16 lobes of the thymus may make a considerable contribution to the increased demand for red blood cells.

While the cyclic erythropoiesis that we have observed in the thymus of adult *Quelea quelea* has not previously been reported, this does not necessarily mean that it is exceptional for there has been no detailed study of the thymus of any other wild bird or mammal and for various reasons it may be expected not to occur in captive, domestic or laboratory animals.

MARION D. KENDALL*
P. WARD

Centre for Overseas Pest Research,
College House, Wrights Lane,
London W8 5SJ

Received October 30, 1973; revised February 21, 1974.

* Present address: Department of Anatomy, St Thomas's Hospital Medical School, Lambeth Palace Road, London SE1 7EH.

- ¹ Romanoff, A. L., *The avian embryo. Structural and Functional Development* (Macmillan, New York, 1960).
- ² Ham, A. W., *Histology* (Lippincott, Philadelphia and Toronto, 1969).
- ³ Romer, A. S., *The Vertebrate Body* (Saunders, Philadelphia and London, 1966).
- ⁴ Ward, P., *Ibis*, **113**, 275 (1971).
- ⁵ Hohn, E. O., *J. exp. Biol.*, **241**, 184 (1947).
- ⁶ Hohn, E. O., *Can. J. Biochem. Physiol.*, **34**, 90 (1956).
- ⁷ Ward, P., and D'Cruz, D., *Ibis*, **110**, 203 (1968).
- ⁸ Anderson, W. L., *Condor*, **72**, 205 (1970).
- ⁹ Yoffey, J. M., and Courtice, F. C., *Lymphatics, Lymph and the Lymphomyeloid Complex* (Academic Press, London, 1970).
- ¹⁰ Terni, T., *Arch. Ital. anat. embriol.*, **21**, 5 (1924).
- ¹¹ Albert, S., Wolf, P., and Pryjma, I., *J. reticuloendothel. Soc.*, **2**, 30 (1965).
- ¹² Sandler, J. A., and Bickerstaff, L. K., *Can. J. Zool.*, **45**, 253 (1967).
- ¹³ Weidenreich, F., *Münch. med. Wschr.*, **58**, 2601 (1912).
- ¹⁴ Weill, P., *Arch. mikr. Anat.*, **83**, 305 (1913).
- ¹⁵ Harland, M., *Anat. Rec.*, **77**, 247 (1940).
- ¹⁶ Sin, Y. M., and Sainte-Marie, G., *Br. J. Haemat.*, **11**, 613 (1965).
- ¹⁷ Sin, Y. M., and Sainte-Marie, G., *Br. J. Haemat.*, **11**, 624 (1965).
- ¹⁸ Pease, C. D., *Blood*, **11**, 501 (1956).
- ¹⁹ Carsairs, K., *Lancet*, **i**, 829 (1962).
- ²⁰ McKinney, A. A., Stohlmann, F., and Brecher, G., *Blood*, **19**, 349 (1962).
- ²¹ Abe, K., Sasaki, K., and Ito, T., *J. Anat.*, **115**, 393 (1973).

²² Ward, P., *Ibis*, **114**, 444 (1972).

²³ Doan, C. A., Cunningham, R. S., and Sabin, F. R., *Contr. Embryol.*, **16**, 163 (1925).

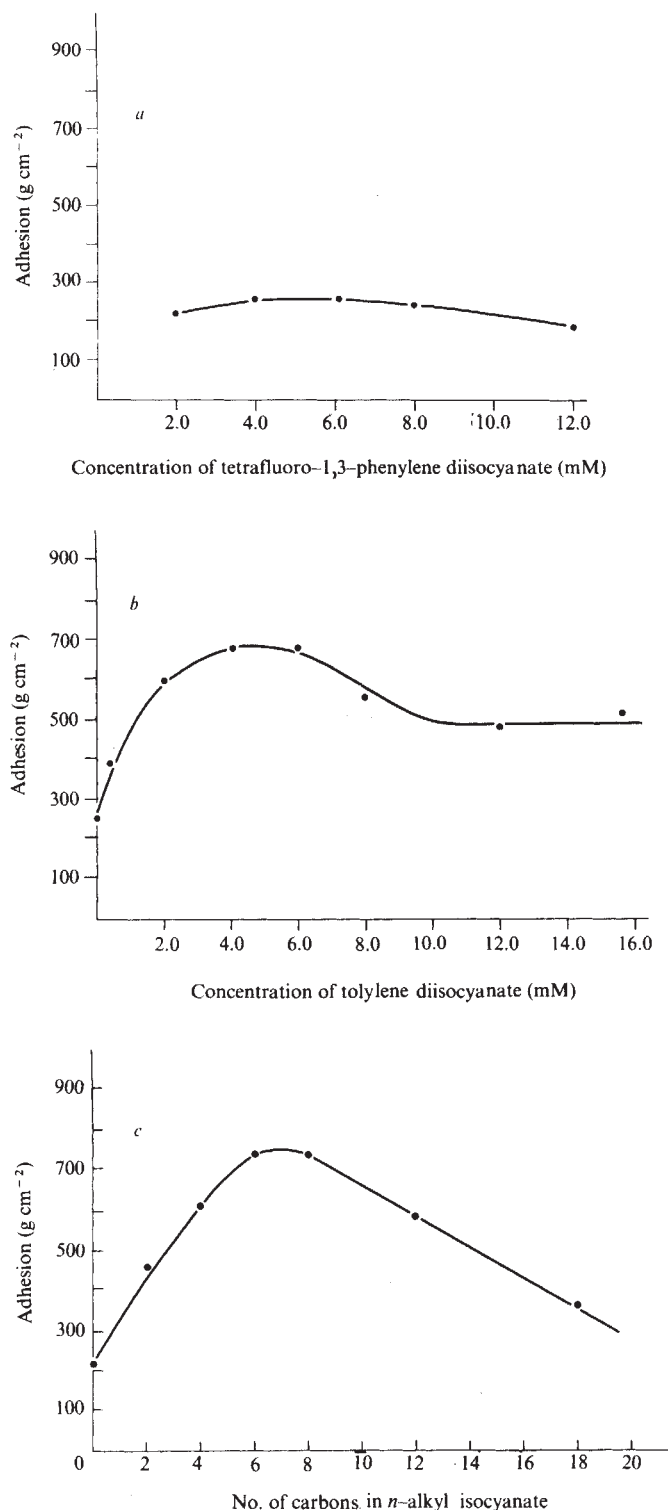
²⁴ Weinberg, S. R., Siegel, C. D., and Gordon, A. S., *Anat. Rec.*, **175**, 7 (1972).

Evidence of hydrophobic interaction in adhesion to tissue

Reaggregation and agglutination of cells *in vitro* have served extensively as model systems in the evaluation of various adhesion phenomena that occur *in vivo*. Such studies have revealed factors that influence cell aggregation^{1–3}, but the mechanism involved at the molecular level has not been investigated fully. In the agglutination of trypsin-treated and virus-transformed cells by concanavalin A, intercellular cross-linking of receptors by the plant lectin is believed to be necessary after ionic repulsion^{4,5}. The adhesion of cells may not, however, depend exclusively on the formation of cross links⁶. Hydrophobic interaction is known to be involved in the aggregation of cell surface components⁷, in the solution behaviour of proteins⁸ and in adsorption⁹, but its contribution to adhesion involving a biological surface has not been considered. This is chiefly because of the difficulty of demonstrating experimentally, by conventional techniques, that hydrophobic interaction can indeed contribute to any significant extent and result in adhesion. I describe here the use of a reactive polyurethane prepolymer to evaluate the conditions required for adhesion to the surface of connective tissue due to hydrophobic interaction.

Castor oil was mixed with the extremely reactive tetrafluoro-1,3-phenylene diisocyanate to produce a syrupy polyurethane prepolymer¹⁰ which contained potentially reactive isocyanate groups¹¹. When this preparation was applied to a wound on the skin of a rat (see legend to Fig. 1), further reaction was initiated by the moisture present, and the prepolymer solidified in 0.3–2.0 min. If hydrophobic interaction was adequate, adhesion should have developed after solidification of the prepolymer. Even with various combinations of castor oil and tetrafluoro-1,3-phenylene diisocyanate, however adhesion was insignificant (Fig. 1a), and limited to the stratum corneum region of the epidermis. When some tolylene diisocyanate was added to the prepolymer, and the mixture was allowed to polymerise on the tissue *in vivo*, the degree of adhesion changed with the amount added (Fig. 1b). Maximum adhesion was found with 4–6 mM tolylene diisocyanate. Adhesion could also be improved considerably by priming the tissue surface with tolylene diisocyanate. This compound could cross link the prepolymer with tissue during polymerisation. If this was the situation, the observed adhesion could not be attributed to hydrophobic interaction. *n*-Hexyl isocyanate, however, which could not form cross links as a monoisocyanate, effected the same degree of adhesion (Fig. 1b and c) when mixed with the prepolymer. Therefore, the condensation of *n*-hexyl isocyanate with receptors on the tissue surface must have made the tissue surface suitable for adhesion. This conclusion was supported by the observation that the degree of adhesion of the hydrophobic prepolymer changed with the number of carbon atoms in an *n*-alkyl isocyanate (Fig. 1c). Maximum adhesion resulted when there were six to eight carbon atoms in the hydrocarbon chain. This was because higher or lower homologues did not have the proper hydrophilic-to-hydrophobic balance in aqueous media¹² for the isocyanate group to react with tissue and effect maximum adhesion.

Under experimental conditions comparable with those on the tissue surface, isocyanates react only with the primary amino and the sulphhydryl groups in proteins^{13,14}, of which there are few in connective tissue. It is remarkable that the conversion of some of the receptor sites is sufficient to alter



the surface hydrophobic character, and effect adhesion in the presence of other unreacted polar groups on tissue components.

These results show that hydrophobic interaction can result in adhesion on a biological surface. The formation of cross links and the nature of tissue surface components do not seem to be critical factors. The adhesion due to this interaction is regulated by the concentration of the converted receptor sites and their hydrophobic characteristics at the interface.

The influence of hydrophobic interaction has been observed to extend to a distance of several microns in the

Fig. 1 Adhesion of the hydrophobic prepolymer to tissue. Several samples containing 1 mM castor oil, 4 mM tetrahydrofuran, 1 mM pyridine and a silicone surfactant¹⁰ (0.4%, w/w) were prepared. These samples were converted into prepolymer by mixing with tetrafluoro-1,3-phenylene diisocyanate. The concentrations of tetrafluoro-1,3-phenylene diisocyanate, tolylene diisocyanate or *n*-alkyl isocyanate required are indicated on the abscissae. Each prepolymer sample of a particular composition was used to close four 1.5 cm stab wounds on the dorsal skin of an anaesthetised Wistar rat. The adhesion of the prepolymer to tissue was assessed on a pneumatic tensiometer¹⁰. Each point on the graphs was the average of the four determinations on the same animal. *a*, Prepolymer samples contained various concentrations of tetrafluoro-1,3-phenylene diisocyanate only. *b*, Various concentrations of tolylene diisocyanate were added to prepolymer samples made with 4 mM tetrafluoro-1,3-phenylene diisocyanate. The horizontal part of the curve was due to cohesive failure of the solidified prepolymer. *c*, Each sample of the prepolymer was prepared with 4 mM tetrafluoro-1,3-phenylene diisocyanate, and then mixed with 4 mM *n*-alkyl isocyanate which had the required number of carbon atoms as indicated.

case of the chemotactic attraction of leukocytes by conjugated proteins^{15,16}. On the surface of unassociated or disassociated cells, requirements for adhesion due to strong hydrophobic interaction, as I have established here, can be more easily achieved by diffusion^{5,17} and configurational changes^{15,18} of surface components. Therefore the contribution of hydrophobic interaction should be given full consideration in studies of adhesion phenomena *in vitro*^{3,5,6}.

I thank the National Research Council of Canada for partial support.

P. Y. WANG

*Institute of Biomedical Engineering,
University of Toronto,
Toronto M5S 1A4*

Received September 28, 1973; revised February 5, 1974.

- Humphreys, T., *Symp. zool. Soc. Lond.*, **25**, 325 (1970).
- Pessac, B., and Defendi, V., *Nature new Biol.*, **238**, 13 (1972).
- Curtis, A. S. G., and de Sousa, M., *Nature new Biol.*, **244**, 45 (1973).
- Nicolson, G. L., *Nature new Biol.*, **239**, 193 (1972).
- de Petris, S., Raff, M. C., and Mallucci, L., *Nature new Biol.*, **244**, 275 (1973).
- Wilkins D. J., Ottewill, R. H., and Bangham, A. D., *J. theor. Biol.*, **2**, 176 (1962).
- Singer, S. J., and Nicolson, G. L., *Science*, **175**, 720 (1972).
- Dandliker, W. B., and de Saussure, V. A., in *The chemistry of biosurfaces*, (edit. by Hair, M. L.), **1**, 25 (Dekker, New York, 1971).
- Hansch, D., and Helmer, F., *J. Polym. Sci.*, **6**, 3295 (1968).
- Llewellyn-Thomas, E., Wang, P. Y., and Vinals, J. N., *Biomat. Med. Dev. Art. Org.*, **1**, 507 (1973).
- Saunders, J. H., and Frisch, K. C., *Polyurethane: II. technology*, 611 (Interscience, New York, 1964).
- Becher, P., *Emulsions: theory and practice*, 242 (Reinhold, New York, 1965).
- Schick, A. F., and Singer, S. J., *J. biol. Chem.*, **236**, 2477 (1961).
- Gaunt, W. E., and Wormall, A., *Biochem. J.*, **30**, 1915 (1936).
- Wilkinson, P. C., and McKay, I. C., *Int. Arch. Allergy*, **41**, 237 (1971).
- Wilkinson, P. C., and McKay, I. C., *Eur. J. Immun.*, **2**, 507 (1972).
- Frye, L. D., and Edidin, M., *J. Cell Sci.*, **7**, 319 (1970).
- Yamada, T., and Yamada M., *Nature*, **244**, 297 (1973).

H₂-receptors in peripheral blood vessels

THE recent observation that certain histamine responses which were resistant to antihistaminic blockade may now be abolished

by the newly synthesised compounds burimamide (N-methyl-N¹-[4-(4/5)-imidazolyl] butyl] thiourea) and metiamide (N-methyl-N¹-[2/(5-methylimidazol-4-yl) methylthio/ethyl] thiourea) (both compounds, Smith, Kline and French) has culminated in the interpretation of histamine action in terms of both H₁- and H₂-receptors¹. Until now the attention of workers in this field has centred upon gastric acid secretion. Our own studies, however, have been directed at the receptor control of the microcirculation and we have previously reported

variation in clearance rate, the method has provided acceptable reproducibility with repeat studies on the same joint¹.

These studies were carried out on adult dogs weighing between 22 and 32 kg using a standard anaesthetic and experimental technique.

Both agonist and antagonist drugs were injected intrarticularly and various doses of each drug (histamine 1–2.5 µg, metiamide 50–1,000 µg and mepyramine 500 and 1,000 µg were used in these studies.

Table 1 ¹³³Xe clearance half life ($T_{\frac{1}{2}}$) values with histamine (H) alone and histamine following H₁ antagonist mepyramine (Mep) given in increasing Mep:H dose ratios

Histamine			Histamine response following mepyramine with various dose ratios Mep:H					
Pre	Post	(µg)	100:1		500:1		1,000:1	
			Pre	Post	Pre	Post	Pre	Post
35	16	(1.0)	60	22	73	25	48	15
25	16	(1.0)	38	18	39	13	73	24
68	18	(1.0)	55	31	32	21	36	23
42	16	(1.0)	150	37	96	51	32	18
68	30	(1.0)					43	23
64	33	(2.5)					66	18
62	35	(2.5)					47	36
50	13	(2.5)						
61	14	(2.5)						
34	16	(2.5)						
Mean	51	21	76	27	60	28	49	22
s.e.m.	5.0	2.7	25	4	15	8	6	3
Mean change	61%		60%		53%		51%	

failure of conventional antihistaminics to block histamine responses in our experimental model². Here we report results obtained in the canine diarthrodial joint which demonstrate the abolition of histamine responses by H₂ and not by H₁-blocking compounds and thus supporting the conclusion that H₂-receptors are involved in the control of peripheral blood vessels.

The clearance rate of radioactive xenon (¹³³Xe) from a diarthrodial joint following intraarticular injection affords an

The relationship between agonist and antagonist was studied in each experiment by injecting first the antagonist followed 10–20 min later by histamine.

Histamine was supplied as histamine acid phosphate (1 mg ml⁻¹ (Evans Medical)), mepyramine as mepyramine maleate (50 mg ml⁻¹) (Anthisan, May and Baker) and metiamide was obtained as a powder from Smith, Kline and French Laboratories. All drug dilutions were with isotonic 0.9% sodium chloride or sterile water.

Table 2 ¹³³Xe clearance half life ($T_{\frac{1}{2}}$) values with metiamide (Met) alone and with histamine following metiamide given in increasing Met:H dose ratios

Metiamide alone			Histamine response following metiamide (Met) in increasing dose ratios (Met:H)							
Pre	Post	(ug)	< 200:1		200:1		500:1		1000:1	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post
115	115	(25)	115	29	20	12	31	31*	39	49*
20	20	(50)	22	11	38	24	47	47*	28	33*
45	55	(50)	28	13	25	25*	26	26*	150	150*
49	51	(200)	60	21	32	29*	60	58*		
50	50	(360)	150	59	50	25	37	33*		
47	50	(560)	36	15	34	24	35	35*		
40	34	(500)	50	20	48	48*				
24	25	(500)								
35	38	(500)								
60	58	(1000)								
150	150	(1000)								
Mean	57	59	66	24	35	28	39	38	72	77
±										
s.e.m.	12	12	18	6	4	4	5	5	39	37
Mean change	1%		59%		19%		0%			

*Indicates instances of complete H₂-receptor blockade.

indirect measure of synovial tissue perfusion^{3,4}. Less than 0.2 ml of the gas dissolved in sterile 0.9% sodium chloride was injected into the diarthrodial joints of anaesthetised adult dogs and the clearance followed by means of a thallium-activated sodium iodide scintillation crystal positioned above the joint and connected to a photomultiplier, pulse height analyser and ratemeter recording on a Rikadenki chart recorder. From a semilogarithmic plot of the count rate the half life ($T_{\frac{1}{2}}$) (min) of the clearance rate was calculated. This technique has been used in the past to demonstrate the presence of adrenergic and cholinergic receptors in the synovial microvasculature, and although there is considerable individual

The results are shown in Tables 1 and 2. Histamine consistently increased the xenon clearance rate in doses of 1 µg and 2.5 µg (Table 1). This response was not abolished by mepyramine maleate in any concentration ratio. Metiamide alone produced no consistent change in xenon clearance rate (Table 2) but completely abolished the action of histamine at dose ratios of 500:1 and 1,000:1 (Met:H). Variable responses were obtained at the ratio of 200:1 and the histamine vasodilator response was clearly apparent at lower concentration ratios.

The abolition of vasodilatation induced by histamine in peripheral blood vessels by metiamide and not by

mepyramine maleate strongly indicates that the effect of histamine in this experimental model is an H_2 -mediated response. Other workers have documented involvement of both H_1 and H_2 -receptors in larger blood vessels^{5,6}. Reed *et al.*⁷ have documented H_2 -receptor abolition of vasodilatation induced by histamine in gastric mucosa using the amidopyrine technique and our results using a radioactive tracer clearance technique are in accord with theirs. The implications of lipid soluble inert gas clearance rates and their relationship to tissue perfusion have previously been discussed⁴. The evidence supports the contention that large changes in clearance rates, produced pharmacologically, may be interpreted as an alteration in tissue perfusion. The expression of results in terms of absolute flow rate per tissue volume, however, is more contentious.

If, as these results indicate, histamine H_2 -receptors are implicated in the control of peripheral blood vessels, a rational explanation is available for the first time for the failure of conventional antihistamine to completely reverse the effect of histamine on systemic blood pressure responses⁸. In addition, the potential interference in vascular responses mediated by histamine offered by metiamide offers an important new approach to the therapy of inflammatory joint disease. It is also conceivable that these methods may be applicable to the study of the relationship between hydrogen ion secretion and peripheral tissue perfusion in diarthrodial joints and in gastric mucosa⁷ and we are currently exploring these possibilities.

We thank Dr M. Bloch and Smith, Kline and French Ltd for advice and for providing the drug metiamide and to the Wellcome Surgical Research Institute, Dr M. Harper and J. G. Littlejohn, for providing facilities and assistance. Financial assistance was provided by the Arthritis and Rheumatism Council (Great Britain).

D. M. GRENNAN
P. J. ROONEY
E. GILBERTSON
W. CARSON DICK

*The Centre for Rheumatic Diseases and
University Department of Medicine, Royal Infirmary;
The Wellcome Surgical Research Institute;
and the Department of Veterinary Surgery, Glasgow;
University Veterinary Hospital, Bearsden, Glasgow.*

Received February 21, 1974.

- ¹ Black, T. W., Duncan, A. M., Durant C. T., Ganellia, C. R. and Parsons, E. M., *Nature*, **236**, 385-390 (1972).
- ² St Onge, R. A., and Dick, W. C., *Modern Trends in Rheumatology*, 2 (edit. by Hill, A. G. S.) **5**, (Butterworths, London, 1970).
- ³ Dick, W. C., *et al.*, *Ann. rheum. Dis.* **29**, 131 (1970).
- ⁴ Dick, W. C., *Seminars in Arthritis and Rheumatism*, **1**, 301 (1972).
- ⁵ Powell J. R., and Brody, M. J., *Int. Symp. on H_2 Receptor Antagonists*, London, (edit. by Wood, C.) **137**, (1973).
- ⁶ Glover, W. E., Carroll, P. R., and Latt, N., *Int. Symp. H_2 -Receptor Antagonists*, London, (edit. by Wood, C.) **169** (1973).
- ⁷ Reed, J. D., Smy J. R., Venables, C. W., and Harris, D. W., *Int. Symp. on H_2 -Receptor Antagonists*, London, (edit. by Wood, C.) **231** (1973).
- ⁸ Douglas, W. W., in *The Pharmacological Basis of Therapeutics* (edit. by Goodman, L. S., and Gilman, A.) 615-627 (McMillan, New York, 1965).

Binding of concanavalin A and ricin to synaptic junctions of rat brain

THE synaptic junction is the intercellular contact where bioelectrical impulses are transferred and transformed by neurotransmitters. The molecular mechanism of impulse transformation is still largely unknown. To understand this process, a detailed knowledge of the structure of the synaptic apparatus is essential. The available information

on the morphology and cytochemistry of synapses has recently been reviewed. To arrive at a more precise morphological characterisation of the synaptic junction with respect to carbohydrate moieties^{2,3}, we investigated the capacity of the plant lectins concanavalin A (con A) and ricin, labelled with ferritin⁴, to bind to isolated synaptosomes (that is, pinched-off axon terminals) and synaptic junctions.

A crude mitochondrial pellet obtained from the brains of adult male Sprague-Dawley rats was further fractionated according to the technique of Levitan *et al.*⁵ to isolate synaptosomal membranes. This fraction was treated with the detergent Triton X-100 at a concentration of 4 mg ml⁻¹ (protein⁶ and Triton X-100 in a ratio of 1:1 by weight), as described by Cotman *et al.*⁷. The resulting suspension was layered on a discontinuous sucrose gradient to isolate the synaptic junctions⁷.

Concanavalin A (Calbiochem) was coupled to ferritin (Schwarz/Mann) with glutaraldehyde (Polysciences) at a molar ratio of 1.0 in the presence of 0.2 M α -methylglucoside in phosphate buffered saline (PBS) at pH 7.3-7.5 (ref. 8). Ferritin-con A and con A were isolated by affinity chromatography on Sepharose G-200 (Pharmacia) and eluted with 0.1 M α -methylglucoside. Ferritin-labelled con A was then separated from unreacted con A by density gradient centrifugation⁹. Ricin was similarly coupled to ferritin and purified according to the same procedure with the exception that Sepharose 4B gel (Pharmacia) and galactose were used instead of Sephadex and α -methylglucoside^{10,11}.

The crude mitochondrial and the synaptic junctional fractions were incubated in 0.2-0.5 ml ferritin-con A 1-2 mg ferritin ml⁻¹ in PBS molar ratio ferritin: con A about 1:3 or in ferritin-ricin (0.4-0.8 mg ferritin ml⁻¹ in PBS, molar ratio ferritin: ricin about 1:3) at 20° C for 1 h. As a control for the specificity of the lectin binding, samples were also incubated in the presence of 0.2 M inhibitor (α -methylmannoside for con A, galactose for ricin). After the incubation, the suspensions were diluted 20-50-fold with PBS, centrifuged at 10,000g for 15 min and washed twice with PBS. The sediments were fixed in 2.5% buffered¹² glutaraldehyde (Polysciences) for 30 min at 20° C, followed by 1% buffered¹³ osmium tetroxide for 1.5 h at 4° C, dehydrated through graded acetones and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate.

We found that of all the synaptic elements investigated the synaptic cleft had the greatest binding capacity for both ferritin-con A (Fig. 1a, b, c) and ferritin-ricin (Fig. 1d); the latter, however, bound to a lesser extent than ferritin-con A. In all control experiments, the binding of lectins was completely inhibited by the presence of the appropriate hapten inhibitor. Compared with intact synaptosomes (Fig. 1a), the cleft region of isolated synaptic junctions (Fig. 1b, c) was considerably more densely coated with labelled con A. Except in the synaptic cleft region, staining of the synaptic junction was negligible. Treatment with Triton, which removes a large part of the presynaptic membrane material from synaptosomes, seems to result in extensive exposure of binding sites located in the cleft region (Fig. 1b, c, d); the penetration of ferritin-labelled con A into the synaptic cleft of intact synaptosomes is obviously restricted (Fig. 1a). Furthermore, the extent to which ferritin-con A binds does not depend on the presence of an intact postsynaptic membrane (Fig. 1b, c).

From these findings we come to the following conclusions. (1) The synaptic cleft contains a high density of con A receptors (believed to be mannosyl or glucosyl end-groups¹³) and an appreciable number of ricin receptors (believed to be galactose end-groups). (2) The consistency of the cleft material in intact synaptosomes is such as to allow the penetration of the comparatively large ferritin-con A molecules (about 150 Å in diameter^{13,14}) (Fig. 1a). After fixation

of the synaptosomes with glutaraldehyde, the accessibility of lectin-binding sites in the synaptic cleft is abolished¹⁵. (3) The lectin-binding carbohydrate groups are firmly bound to the cleft region, since they are insensitive to the detergent treatment; they could be anchored within that part of the postsynaptic web which is in contact with the synaptic cleft. In such an arrangement, the connecting molecular links would run through the postsynaptic membrane. Alternatively, the removal of the postsynaptic membrane by the detergent could simply expose additional binding groups.

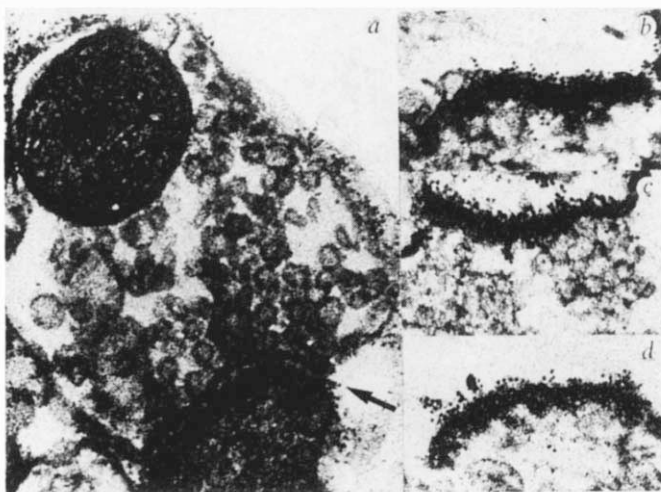


Fig. 1 *a*, Synaptosome labelled with ferritin-con A; ferritin-con A is dispersed over the membrane of the axon terminal (recognisable by the synaptic vesicles) and the postsynaptic membrane. Ferritin-con A can penetrate the synaptic cleft (arrow) and bind there specifically ($\times 27,000$); *b*, Synaptic junction stained with ferritin-con A; postsynaptic membrane dissolved by Triton X-100 ($\times 34,200$). *c*, Synaptic junction stained with ferritin-con A; postsynaptic membrane intact ($\times 34,200$). *d*, Synaptic junction stained with ferritin-ricin; postsynaptic membrane dissolved by Triton X-100 ($\times 34,200$).

The high density of con A-receptors at the synaptic junctions was subsequently exploited for their purification on con A-Sepharose gel (Pharmacia). In preliminary experiments, a three-fold enrichment (as judged by morphometric analysis of electron micrographs) of synaptic junctions compared with the final material from conventional procedures (density gradient) was obtained.

We thank W. Schweizer and J. Heid for experimental assistance and Drs S. de Petris and G. L. Nicolson for suggestions concerning the coupling of concanavalin A to ferritin. The ricin was a gift from Professor H. J. Horstmann, University of Erlangen, Germany.

HELMUT BITTIGER

Research Laboratories,
Pharmaceuticals Division,
Ciba-Geigy,
CH-4002 Basel, Switzerland

HANS PETER SCHNEBLI

Friedrich Miescher Institut,
Basel, Switzerland

Received January 15, 1974.

¹ Pfenninger, K. H., *Progr. Histochem. Cytochem.*, **5**, 1-86 (Gustav Fischer Verlag, Stuttgart, Portland, 1973).

² Rambourg, A., and Leblond, C. P., *J. Cell Biol.*, **32**, 27-53 (1967).

³ Meyer, W. J., *J. Cell Biol.*, **43**, 92a (1969).

⁴ Hirano, H., Parkhouse, B., Nicolson, G. L., Lennox, E. S., and Singer, S. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2945-2949 (1972).

⁵ Levitan, J. B., Mushynski, W. E., Ramirez, G., *J. biol. Chem.*, **247**, 5376-5381 (1972).

⁶ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).

⁷ Cotman, C. W., and Taylor, D., *J. Cell Biol.*, **55**, 696-711 (1972).

⁸ Stobo, J. D., and Rosenthal, A. S., *Expl Cell Res.*, **70**, 443-447 (1972).

⁹ de Petris, S., and Raff, M. C., *Eur. J. Immun.*, **2**, 523-535 (1972).

¹⁰ Nicolson, G. L., and Blaustein, J., *Biochim. biophys. Acta*, **266**, 543-547 (1972).

¹¹ Tomita, M., Kurokawa, T., Onozaki, K., Ichiki, N., Osawa, T., and Ukita, T., *Experientia*, **28**, 84-85 (1972).

¹² Millonig, G., *Proc. fifth int. Congr. Electron Microscopy, Philadelphia* (edit. by Breese, S. S., jun.), **2**, 8 (Academic Press, New York, 1962).

¹³ Sharon, L., and Lis, H., *Science*, **177**, 949-959 (1972).

¹⁴ Crichton, R. R., *Angew. Chem.*, **85**, 53-62 (1973).

¹⁵ Matus, A., de Petris, S., and Raff, M. C., *Nature new Biol.*, **244**, 278-280 (1973).

Toxins of *Vibrio cholerae* and *Escherichia coli* stimulate adenyl cyclase in rat fat cells

RAT epididymal fat cells respond to enterotoxins of *Vibrio cholerae* and *Escherichia coli* with increased membrane adenyl cyclase activity and glycerol release¹⁻³. Cholera toxin increases adenyl cyclase activity in many other tissues and cells, including intestine, leukocytes, immunocytes, platelets and liver⁴⁻⁶. Here we report studies on the response of fat cells to these bacterial toxins and their effect on membrane adenyl cyclase activation by adrenaline.

E. coli toxin was prepared from a Millipore filtrate of an 18h culture of a strain of *E. coli* previously isolated from a patient with nonvibrio cholera^{7,8} grown in medium containing the following concentrations of materials in g l⁻¹: 20 g Bacto-Difco casamino acids, 1.5 g Bacto-Difco yeast extract, 2.5 g NaCl, 8.71 g K₂HPO₄ (0.05 M), and 1.0 ml Trace salts solution (containing 50 g l⁻¹ MgSO₄, 5.0 g l⁻¹ MnCl₂, 5.0 g l⁻¹ FeCl₃ dissolved in 0.001 N H₂SO₄). The mixture was adjusted to pH 8.5 with 0.1 N NaOH. Cultures were grown with shaking 500 ml in a 2.5 l low-form flask. After Millipore filtration, the TRY culture media supernatant was concentrated by UM-10 Diaflow filtration to a final concentration of 10 mg ml⁻¹. Highly purified *V. cholerae* toxin was prepared by Finkelstein, according to a method previously described⁹. Both toxins were tested for their ability to produce fluid loss in duodenal loops of mongrel dogs¹⁰. Fat cells were isolated from the epididymal fat pads of male Sprague-Dawley rats by treatment with collagenase (4 mg ml⁻¹) for 1 h, filtration through silk screen, and subsequent washing three times with buffer. All incubations were carried out in Krebs-Ringer phosphate buffer, pH 7.2, with albumin (3.63 g per 120 ml) while shaking at 60 r.p.m. in a 37°C water bath. Toxins were added at time zero at concentrations of 1 mg ml⁻¹ (*E. coli*) and 1 µg ml⁻¹ (*V. cholerae*) to fat cells 0.5 g 1.0 ml. At the end of 10 min, all samples were diluted with buffer to final volume of 5 ml (except the 5 min point which was diluted at 5 min). Incubation was terminated by centrifugation of cells and media for 1 min at 1,500 r.p.m. and removal of infranatant buffer for glycerol determination¹¹. Fat cells were lysed by exposure to chilled (4°C) distilled water and mixing twice on a vortex shaker. Membranes were isolated in a pellet by centrifugation at 12,000g for 10 min at 4°C. Pellets were resuspended in cold Tris-MgCl₂ buffer (75 mM Tris, 25 mM MgCl₂, pH

8.0) adjusted to make a protein concentration of 25–100 μg in 55 μl of total reaction mixture. Adenyl cyclase activity was subsequently measured by conversion of ^{32}P -ATP to ^{32}P -cyclic AMP according to the method of Krishna¹². The responsiveness of membrane adenyl cyclase to adrenaline was evaluated by adding 5.5 μM adrenaline to the adenyl cyclase assay of membranes from control cells and exposed cells exposed to toxin.

The stimulation of membrane adenyl cyclase by *E. coli* toxin was at its maximum (642 ± 269 pmol cyclic AMP per mg protein per 10 min increase over control) by 30 min, falling to control levels within 60 min (Fig. 1a). The response to cholera toxin, however, was not measurable until after 60 min and continued to rise to a plateau of 1244 ± 121 pmol cyclic AMP mg^{-1} protein per 10 min increase over control adenyl cyclase activity at 3–4 h. The action of a single dose of cholera toxin on gut cells is known to continue for at

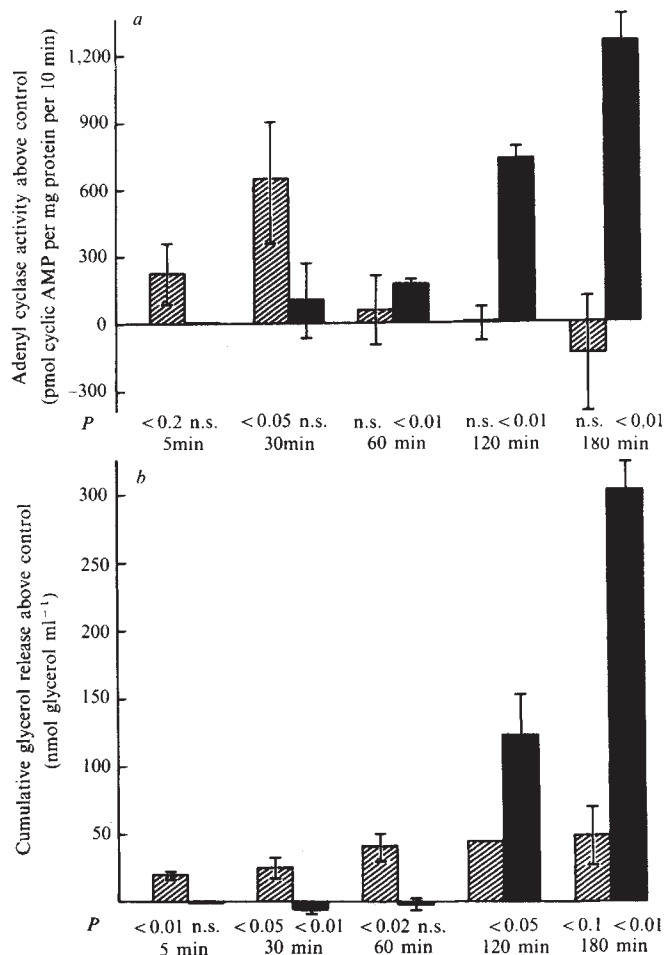


Fig. 1 Time course of the response of isolated rat epididymal fat cells to *E. coli* (▨) or *V. cholerae* (■) enterotoxins (9195 and 1071, respectively) measured by adenyl cyclase activity in pmole cyclic AMP per mg protein per 10 min above control values (a), or cumulative glycerol release in nmol ml^{-1} above controls (b). Values expressed as mean $\pm$ s.e.m.

least 12 h but measurement of duration is limited in our system by viability of isolated fat cells⁴. Statements about the effects after 3 h are not therefore possible.

Glycerol release in response to cholera toxin began after 60 min and continued beyond 3 h. Neither the adenyl cyclase activation nor the glycerol release induced by cholera toxin was significantly altered by the five-fold dilution of cells and toxin after the initial 10 min incubation, indicating rapid and strong binding of toxin to cell. A signi-

ficant lipolytic response to *E. coli* toxin was seen at 5 min. This activity was heat labile (boiling for 10 min). By 3 h a total 48 nmol ml^{-1} of glycerol had been released. Cells which were not diluted as described previously and were exposed continuously to 1 mg ml^{-1} of *E. coli* toxin released 138 nmol ml^{-1} of glycerol by 3 h. This difference in response to the same initial concentration and total dose of toxin indicates a more transient or less avid interaction with cells. A similar action of *E. coli* toxin on canine intestine has been reported¹⁰. Preliminary experiments with fat cells indicate that washing cells previously exposed to toxin has no effect on response to cholera toxin, but washing cells exposed to *E. coli* toxin at 5 min can prevent a further rise in adenyl cyclase activity and glycerol release.

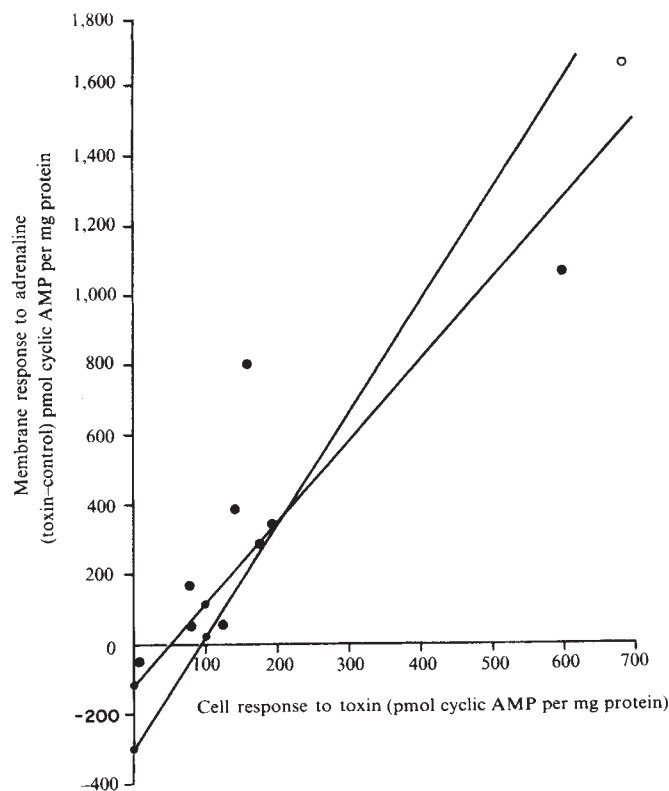


Fig. 2 Responsiveness to adrenaline of membranes of cells previously exposed to *E. coli* ($n=14$) or *V. cholerae* ($n=1$) toxins. ●, Cells exposed to *E. coli* toxin; ○, cells exposed to cholera toxin. $R=0.86$ $P < 0.01$. The regression lines indicate the slopes at 95% confidence limits.

In spite of the marked difference in the time course of adenyl cyclase stimulation by these two bacterial toxins, both have a significant effect on the responsiveness of membrane adenyl cyclase to further stimulation by adrenaline. Exposure of membranes to 5.5 μM adrenaline at the time of adenyl cyclase assay resulted in a significantly greater increase in adenyl cyclase activity in membranes previously exposed to either toxin than in control membranes (Fig. 2). This heightened response was greatest in membranes showing the greatest response to either toxin.

Enterotoxins of *E. coli* and *V. cholerae* produce gut fluid loss by similar actions on ion movement across gut mucosa. They each produce an altered ion flux by stimulating adenyl cyclase and thereby increasing cyclic AMP levels^{10,13}. Each affects tissues other than intestinal mucosa, producing effects compatible with increased levels of cyclic AMP. There is also a close antigenic relationship between the heat-labile components of *E. coli* and enterotoxins demonstrated by immunological cross reactivity with specific antisera¹⁴.

Although the relationship to toxin receptor and regulator to the hormonal receptor and regulator is not known, other studies have indicated that toxin action does not interfere with normal channels of hormone action^{1,16}. Here, we have demonstrated not only a lack of interference, but also a marked facilitation by toxin of cellular response to a hormonal stimulus. The enhanced responsiveness of cell membrane adenylyl cyclase to adrenaline is related directly to the response of cells to toxin. Thus, stimulation of whole cells by toxin sensitises their membranes to subsequent catecholamine challenge. The mechanism of this enhanced hormonal responsiveness is not clear.

V. cholerae infections are restricted to the gut lumen and the action of its enterotoxin is only on intestinal epithelium. It will be of interest to determine whether the sensitisation of intestinal epithelium to hormonal stimuli plays a role in cholera in addition to the known direct action of cholera toxin. The *E. coli* enterotoxin(s), however, may not be restricted in their action to the intestine as *E. coli* frequently causes tissue infections and septicaemia. Should enterotoxin production occur in the blood stream or other tissues, a variety of biological effects might be expected based both on the direct stimulation of adenylyl cyclase in the involved tissues and on enhanced responsiveness to normal hormonal stimuli. Such effects are seen when cholera toxin is given parenterally to animals¹⁷. Some effects such as hypersensitivity to catecholamines have already been observed and attributed, perhaps erroneously, to endotoxin¹⁸.

This work was supported by grants from the United States-Japan Cooperative Medical Science Program administered by the National Institutes of Health of the Department of Health, Education and Welfare. We wish to thank Julie Yang for technical assistance.

ERIK L. HEWLETT
RICHARD L. GUERRANT
DOYLE J. EVANS, jun.
WILLIAM B. GREENOUGH III

Departments of Medicine,
The Johns Hopkins University
School of Medicine and Hospital,
and The Baltimore City Hospitals,
Baltimore, Maryland

Received March 7; revised December 27, 1973.

- ¹ Vaughan, M., Pierce, N. F., and Greenough, W. B. III., *Nature*, **226**, 658 (1970).
- ² Hewlett, E. L., and Greenough, W. B. III., *Clin. Res.*, **19**, 459 (1971).
- ³ Evans, D. J. Jr., Chen, L. C., Curlin, G. T., and Evans, D. G., *Nature new biol.*, **236**, 137 (1972).
- ⁴ Sharp, G. W. G., and Hynie, S. *Nature*, **229**, 266 (1971).
- ⁵ Zieve, P. D., Pierce, N. F., and Greenough, W. B. III., *Johns Hopkins med. J.*, **129**, 299 (1971).
- ⁶ Lichtenstein, L. M., Henney, C. S., Bourne, H. R., and Greenough, W. B. III., *J. clin. Invest.*, **52**, 691 (1973).
- ⁷ Evans, D. G., Evans, D. J., jun., and Pierce, N. F., *Bact. Proc.*, (in the press).
- ⁸ Lindenbaum, J. L., Greenough, W. B. III., Benenson, A. S., Oseasohn, R., Risvi, S., and Saad, A., *Lancet*, **i**, 1081 (1965).
- ⁹ Finkelstein, R. A., and LoSpalluto, J. J., *J. infect Dis.*, **121**, S63 (1970).
- ¹⁰ Guerrant, R. L., Ganguly, U., Casper, G. T., Moore, E. J., Pierce, N. F., and Carpenter, C. C. J., *J. clin. Invest.*, **52**, 1707 (1973).
- ¹¹ Mosinger, B., and Vaughan, M., *Biochim. biophys. Acta*, **144**, 569 (1967).
- ¹² Krishna, G., Weiss, B., and Brodie, B. B., *J. Pharm. exp. Ther.*, **163**, 379 (1968).
- ¹³ Al-Awqati, Q., Wallace, C. K., and Greenough, W. B. III., *J. infect Dis.*, **125**, 00 (1972).
- ¹⁴ Smith, N. W., and Sack, R. B., *J. infect Dis.*, **127**, 164 (1973).
- ¹⁵ Serebro H. A., Iber, F. L., Yardley, J. H., and Hendrix, T. R., *Gastroenterology*, **56**, 506 (1969).
- ¹⁶ Katz, M. S., and Greenough, W. B. III., *Clin. Res.*, **20**, 531 (1972).

¹⁷ Pierce, N. F., Graybill, J. R., Kaplan, M. M., and Bouwman, D. L., *J. lab. clin. Med.*, **79**, 145 (1972).

¹⁸ Zweifach, B. W., Nagler, A. L., and Thomas, L., *J. exp. Med.*, **104**, 881 (1956).

Subterminal motor nerve abnormalities in psychotic patients

THERE is considerable evidence of neuromuscular dysfunction in most patients with all types of the functional psychoses. Examples of such dysfunction are (1) increased activity of skeletal-muscle-type creatine phosphokinase (CPK) in serum in the acute stage of psychosis¹⁻⁴; (2) abnormally large increases in serum CPK activity with a standardised exercise test performed by patients in symptomatic remission⁵; (3) morphological abnormalities in skeletal muscle fibres at biopsy, particularly scattered atrophic fibres and extensive areas of Z-band streaming⁶⁻⁸; and (4) abnormalities of eye movements in tracking a pendulum⁹. The skeletal muscle fibre abnormalities found in psychotic patients are likely to be neurogenic in origin¹⁰.

We now report neurological abnormalities of subterminal motor axons in 21 out of 29 psychotic patients. Our subjects were 16 acute and three chronic schizophrenics, one patient with a paranoid psychosis, four manic-depressives—manic or hypomanic phase—five psychotic depressives and two nonpsychotic hospitalised depressed patients. Diagnoses were made by consensus of two psychiatrists and one social worker without knowledge of the

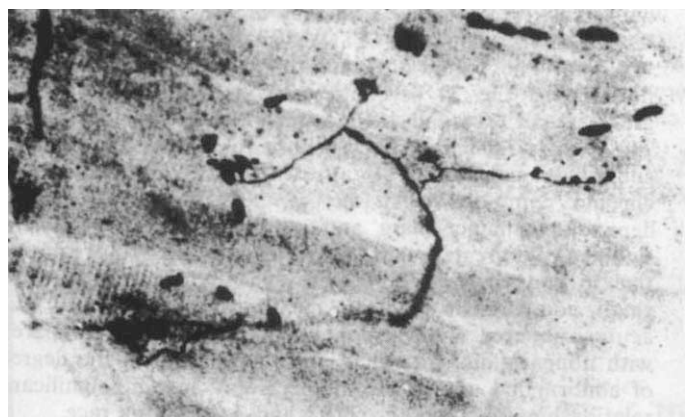


Fig. 1 The subterminal nerve ends in five branches. The two branches on the right end on the same skeletal muscle fibre. The stain was methylene blue, $\times 360$.

biological data. All patients with the diagnosis of schizophrenia satisfied the criteria of the New Haven Schizophrenia Index¹¹. The patient groups consisted of 15 males and 14 females; their median age was 24 (range, 17–45). The control group consisted of three male and three female volunteers who were accepted only after a thorough history revealed no personal or familial medical illness that might affect neuromuscular pathology. Their median age was 23 (range, 22–35).

The peroneus brevis was biopsied under local anaesthesia. The subterminal motor nerves were stained supravitaly with methylene blue¹² and the absolute and functional terminal innervation ratios (ATIR and FTIR respectively)¹³ were determined by carefully following at least 100 sub-

terminal motor nerves selected randomly from all of the slides prepared from each patient. All histological work was performed on a blind basis by coding the slides. To test the reliability of the method, investigators made independent assessments of coded slides. The inter-rater correlation coefficient of six innervation ratios was $r=0.932$ ($P<0.01$). We have also computed the percentage of axons with more than two branches, since such extensive branching has been considered to be diagnostic of denervation and reinnervation. Two other small samples from the same muscle were obtained for histochemical studies and phase microscopy by methods described previously^{6,7}.

TIRs for patients and controls are summarised in Table 1.

Table 1 Absolute and functional terminal innervation ratios

Group	N	Absolute TIR		Functional TIR	
		Mean	s.d. Range	Mean	s.d. Range
Chicago controls	6	1.08±0.049	1.05–1.16	1.07±0.052	1.05–1.16
Controls of Cöers ^{8,12}	56	—	—	1.11±0.05	1.01–1.20
All psychotics	29	1.49±0.46‡	1.00–2.90	1.39±0.34‡	1.00–2.35
All schizophrenics	19	1.46±0.50‡	1.00–2.90	1.36±0.36‡	1.00–2.35
Affective psychoses	9	1.57±0.34‡	1.04–1.93	1.48±0.31‡	1.03–1.87
Neurotic depression	2	1.11±0.01*	1.10–1.12	1.11±0.01*	1.09–1.12

The ATIR is defined as the number of end plates innervated by a given number of axons divided by that number of axons. Similarly, the FTIR is defined as the number of muscle fibres innervated by a given number of axons divided by that number of axons. The mean ATIR and FTIR of the experimental groups were compared with those of the Chicago controls by means of a Mann-Whitney U test, two-tailed: * $P>0.5$; † $P>0.01$; ‡ $P>0.005$.

The mean FTIR of the experimental subjects were compared with those of the controls of Cöers and colleagues¹⁴ using a two-tailed *t*-test: § $P<0.001$; ¶ $P>0.5$.

The TIRs of the six controls were in excellent agreement with those reported by Cöers *et al.*^{13–15}. The 95% upper limit of normal for the ATIR was 1.16; for the FTIR it was 1.15. The TIRs of the two nonpsychotic patients were normal. Of the 29 psychotic patients, 21 had an ATIR greater than 1.16 and 19 had an FTIR greater than 1.15. The mean TIRs for all psychotic patients, and for the subgroups consisting of all schizophrenics (acute and chronic combined) and the affective psychoses (manic-depression, manic phase and psychotic depression), were significantly greater than those of our six controls and the 56 controls of Cöers *et al.*¹⁵. Although groups were small, comparisons of group means gave no evidence that acute compared with chronic status or paranoid compared with nonparanoid status had any relationship to the degree of abnormality of the TIRs. There also were no significant relationships between the TIRs and age, sex or race.

The mean TIRs of the 12 psychotic patients who had received the largest total of phenothiazines (8–270 g) were not significantly greater than those of the 12 patients who had received the smallest doses (0–5.8 g). Increased ratios were found in four of six psychotic patients who had received a total of less than 1 g of chlorpromazine.

The type of branching of the subterminal motor axons in the muscle of the psychotic patients is illustrated in Figs 1 and 2. The 95% confidence limit for the percentage of fibres with three or more branches was 6.2% in the six controls. Cöers *et al.* found a mean of 0.65% of subterminal axons with three or more branches in the palmaris longus and tibialis anterior muscles of 13 controls¹⁸. Fifteen of the twenty-nine psychotic patients had more than 6.2% of axons with three or more branches (range, 6.6–25.4%) with four patients having 20% or more of their axons with three or more branches.

The relationship between the TIRs and the results of the histochemical and phase microscopy studies of muscle fibres

in the psychotic patients will be described in detail elsewhere¹⁷. Here we wish to indicate some important relationships between muscle fibre abnormalities and subterminal motor nerve changes. Three of the psychotic patients with an excessive percentage of fibres with three or more branches had several groups of 50–100 fibres of the same fibre type rather than the usual checkerboard pattern, or atrophic fascicles (Fig. 3). Previous studies have demonstrated a relationship between elevated FTIRs and type grouping¹⁸. The correlation between the FTIR and the number of small angular fibres in the sample taken for histochemistry was highly significant ($r=0.719$, $N=29$, $P<0.01$). On the other hand, there was no correlation between either TIR and the percentage of fibres with extensive areas of Z-band streaming. The TIRs of the 22 psychotic patients with abnormalities in muscle fibre morphology were, however, significantly greater than those of the seven psychotic patients without such abnormalities ($P<0.05$, two-tailed). Since scattered small angular fibres and Z-band streaming have both been described in neuropathic motor disorders^{19–21}, it seems likely that neuronal dysfunction is the basis for both the muscle pathology and the increased subterminal axonal branching in psychotic patients. Additional support for this hypothesis comes from the observation that the ATIR and FTIR were not significantly different in the psychotic patients. This pattern strongly suggests a neurogenic motor disorder, because in primary muscle disease the FTIR remains near unity, while the ATIR increases¹³.

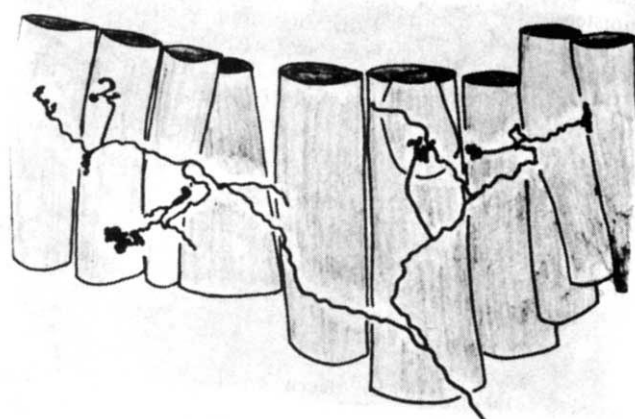


Fig. 2 Camera lucida drawing. The motor nerve can be followed to at least eight recognisable end plates. Several sprouts (branches without end plates) are evident.

In addition to the branches with terminal arborisations, excessive formation of sprouts, or immature branches, was found in 11 of 29 psychotic patients (Fig. 2). Cöers and Woolf have found that sprouting is characteristic of anterior horn cell disease and vitamin B₁₂ deficiency whereas in peripheral neuritis there is little sprouting because the healthy motor neurone somata support the formation of healthy branches rather than sprouts¹³. The presence of sprouting along with fibre type grouping and excessive numbers of small angular fibres, indicates the α -motor neurone as a probable locus of neuropathology in these patients. It seems likely that the effectiveness of branching is one reason why relatively few scattered atrophic fibres are present in the psychotic patient.

These studies therefore show that there are neuropathological changes in patients with the so-called functional psychoses. The significance, if any, of these abnormalities of the motor nerves for motor performance in psychotic patients is an intriguing question. Evidence for impaired psychomotor performance in schizophrenics has been re-

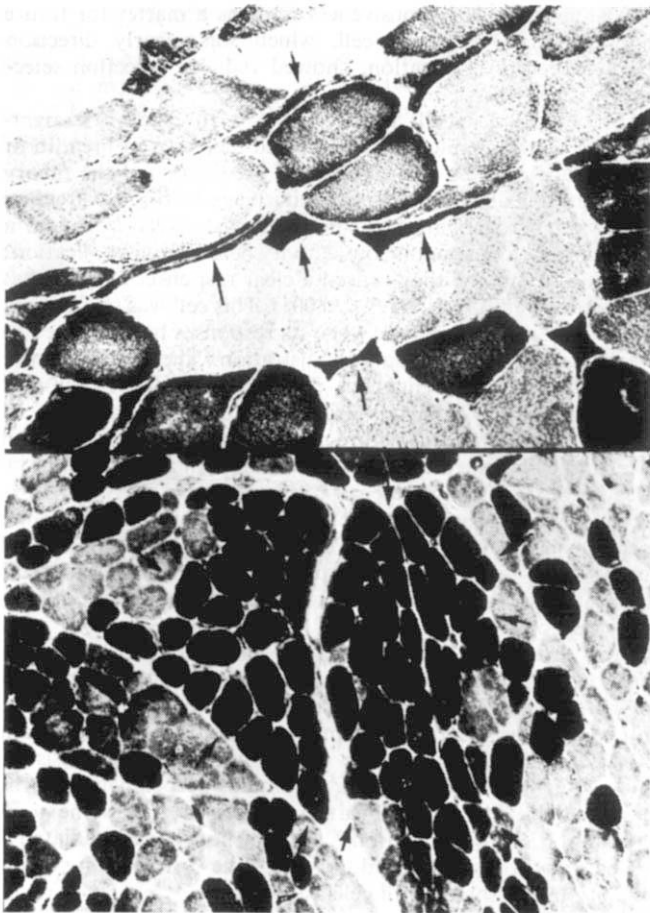


Fig. 3 Top, four small, angular atrophic type I muscle fibres are indicated by arrows. (Nicotinamide adenine dinucleotide-tetrazolium reductase, $\times 167.40$.) Bottom, two groups of type I fibres are indicated by arrows. (Nicotinamide adenine dinucleotide-tetrazolium reductase, $\times 86.80$.)

viewed recently²². It is conceivable that the abnormalities of motor nerves reported here might affect a number of critical fine motor processes such as eye movements, larynx and finger dexterity such that they contributed to the schizophrenics' difficulties in adaptation.

This work was supported by grants from the National Institute of Mental Health, the Department of Mental Health of the State of Illinois and the Aaron Frosch Foundation.

H. Y. MELTZER
J. W. CRAYTON

Department of Psychiatry,
University of Chicago Pritzker School of Medicine and
the Illinois State Psychiatric Institute,
Chicago, Illinois

Received February 6; revised March 21, 1974.

- ¹ Meltzer, H., *Science*, **159**, 1368–1370 (1968).
- ² Meltzer, H. Y., and Moline, R., *Arch. gen. Psychiat.*, **23**, 481–491 (1970).
- ³ Schweid, D. E., Steinberg, J. S., and Sudak, H., *Arch. gen. Psychiat.*, **26**, 263–265 (1972).
- ⁴ Gosling, R., Kerry, R. J., and Owen, G., *Br. med. J.*, **3**, 327–329 (1972).
- ⁵ Meltzer, H., and Moline, R., *Arch. gen. Psychiat.*, **22**, 390–397 (1970).
- ⁶ Meltzer, H. Y., and Engel, W. K., *Arch. gen. Psychiat.*, **23**, 492–502 (1970).
- ⁷ Fischman, D. A., Meltzer, H. Y., and Poppei, R. W., *Arch. gen. Psychiat.*, **23**, 503–515 (1970).
- ⁸ Meltzer, H. Y., McBride, E., and Poppei, R. W., *Neurology Minneapolis*, **23**, 769–780 (1973).
- ⁹ Holzman, P. S., Proctor, L. R., and Hughes, D. W., *Science*, **181**, 179–181 (1973).
- ¹⁰ Meltzer, H. Y., *Arch. gen. Psychiat.*, **27**, 125–132 (1972).

- ¹¹ Astrachan, B. M., Harrow, M., Adler, D., Brauer, L., Schwartz, A., Schwartz, C., and Tucker, G., *Br. J. Psychiat.*, **121**, 529–539 (1972).
- ¹² Evans, R. H., Haynes, J., Morris, C. J., and Woolf, A. L., *J. neurol. neurosurg. Psychiat.*, **33**, 783–785 (1970).
- ¹³ Coërs, C., and Woolf, A. L., *The innervation of muscle* (Blackwell, Oxford, 1965).
- ¹⁴ Reske-Nielsen, E., Coërs, C., and Harmsen, A., *J. neurol. Sci.*, **10**, 369–384 (1970).
- ¹⁵ Coërs, C., Telerman-Toppet, N., and Gérard, J.-M., *Arch. Neurol.*, **29**, 210–214 (1973).
- ¹⁶ Coërs, C., Reske-Nielsen, E., and Hermesen, A., *J. neurol. Sci.*, **19**, 351–356 (1973).
- ¹⁷ Meltzer, H. Y., and Crayton, J. W., *Biol. Psychiat.* (in the press).
- ¹⁸ Morris, C. J., *J. neurol. neurosurg. Psychiat.*, **32**, 440–444 (1969); *J. neurol. Sci.*, **11**, 129–136 (1970).
- ¹⁹ Engel, W. K., in *Proc. fifth Int. Cong. Neuropath.*, Zurich, 1965, 613–624 (Excerpta Medica Foundation, Amsterdam, 1966).
- ²⁰ Shafiq, S. A., Milhorat, A. T., and Gorycki, M. A., *Neurology Minneapolis*, **17**, 934–948 (1967).
- ²¹ Mendell, J. R., and Engel, W. K., *Neurology Minneapolis*, **21**, 358–365 (1971).
- ²² Holzman, P. S., *Psychopharmacologia Berlin*, **24**, 29–41 (1972).

Effects of bicuculline on functions of inhibition in visual cortex

FURTHER to a recent report¹ on the effects of intravenous bicuculline on neurones in the cat visual cortex, we feel that we should present some preliminary results from our own experiments.

In the mammalian cerebral cortex, bicuculline acts as a reversible inhibitor of the action of γ -aminobutyric acid (GABA) (ref. 2), the principal putative inhibitory neurotransmitter³. In the visual cortex, intracortical inhibitory interneurons may act to increase the precision with which a neurone is selectively responsive to a particular visual stimulus, for example, one specific orientation of a moving edge or bar^{4–5}. The direct excitatory input to the cell from the lateral geniculate nucleus, (LGN), may only broadly 'tune' the cell in its responses to orientation, while an inhibitory input (even more broadly tuned for orientation), through intracortical interneurons, could suppress the responses to stimuli away from the optimal orientation^{6–9} (Fig. 1a). This would give improved specificity for one particular orientation at the expense of general decreased responsiveness. According to this theory, then, blockage of intracortical inhibitory neurotransmission, such as with bicuculline, should induce an increase in the breadth of orientation tuning; a proportionately greater increase in responsiveness (Fig. 1a); and, perhaps, a rise in spontaneous discharge if there is tonic activity of inhibitory interneurons¹⁰. Also, bicuculline might reduce the specificity for stimulus length (hypercomplex cells)¹¹, direction and velocity of movement^{8, 10}, and binocular suppressive interactions¹², all of which could depend on intracortical inhibitory interactions.

Pettigrew and Daniels have reported¹ that bicuculline administered intravenously increased the breadth of orientation tuning, responsiveness, and spontaneous activity of complex cells, modified the receptive fields, and sometimes abolished direction selectivity. Hypercomplex cells lost their preference for short bars. Simple cells, however, became less responsive and sometimes more narrowly tuned. Thus, their reported results partially support and partially conflict with the theory described here.

In our experiments, adult cats were paralysed with Flaxedil and artificially ventilated with N_2O/O_2 mixture¹³. Extracellular recordings were made in area 17 (verified by later histology) using glass-insulated tungsten microelectrodes and conventional amplification and display techniques. Quantitative estimations of the characteristics of each cell were made by

averaging the number of spikes given in response to several sweeps of a bright bar of optimum dimensions across the face of an oscilloscope, at many different orientations. Linear regression lines were fitted to these data to form tuning curves¹⁴ (Fig. 1*b* and *c*). Changes in these curves after drug application were assessed, using empirical criteria for their reliability: changes in breadth of tuning of $>15\%$ and in peak response (at the optimal orientation) of $>20\%$ were considered significant¹⁴, while *t* tests were used to assess shifts in spontaneous firing level (Table 1). Several hours were allowed between experiments on cells in the same cat to ensure complete recovery from the drug. The mechanical stability of the preparation for topical application is erratic in some experiments and has meant that few cells have been fully analysed so far.

First, the effects of intravenous bicuculline (0.2 mg kg^{-1} ; K & K Laboratories) were studied on two simple and three complex cells, with essentially negative results. For perhaps a minute after injection, the EEG showed very large slow waves or epileptiform spikes (effects were inconsistent). Data were collected within 13–29 min after injection, and from these an orientation tuning curve was constructed and compared with that obtained before drug application.

No systematic changes were seen (Table 1). Intravenous application of bicuculline has unknown potential actions on neural systems (such as the retina¹⁵, LGN (ref. 16) and reticular system¹⁷), and can cause tachycardia and hypertension¹⁸. Some of the findings of Pettigrew and Daniels¹, especially the consistently decreased responses of simple cells, cannot be easily explained in terms of neural effects within the primary visual cortex alone.

For topical application experiments, the electrode was introduced through a closed perspex chamber cemented over a 3–5 mm diameter craniotomy above the visual cortex. The chamber was filled with warmed saline through long thin tubing to damp down pulsations and the pressure was maintained at 10–20 mm H₂O. The saline could be replaced with 10^{-4}M or 10^{-5}M bicuculline as hydrochloride in saline.

Topical bicuculline broadened the tuning of all three simple cells tested (though one by only 12%) and the other predictions of the theory described above were verified completely in two of these cells (Table 1 and Fig. 1*b*). In two simple cells the receptive fields were plotted again and they had increased three-fold and five-fold in area. The possible correlation of this

with alterations in responsiveness remains a matter for future investigation. One simple cell, which was clearly direction selective before application, showed reduced direction selectivity afterwards.

Of the complex cells tested, the first three gave straightforward results (Table 1). Two showed increases in breadth of tuning. One of these two fulfilled all predictions of the theory under test and also showed a quantitative reduction of direction selectivity; bars moving in the nonpreferred direction caused a 25% inhibition of spontaneous activity before drug application; but with bicuculline they caused a clear response; 24% of that in the preferred direction ($P < 0.001$). This cell was still present 3–3.5 h after drug removal, when its responses had returned to the condition before drug administration. The third complex cell showed no consistent effect of the drug. The fourth complex cell was unusual in having clear inhibitory 'side-flanks' in its orientation tuning curve before application of the drug. Topical bicuculline abolished these side flanks as well as increasing the breadth of tuning and the peak response (Fig. 1*c*).

The next day, a fifth complex cell was found in the same location in the cortex, with very similar receptive fields and tuning curve to the fourth complex cell. Immediately after the application of bicuculline there was actually a decrease in both peak response and breadth of tuning (Table 1), but after 45 min exposure, both parameters had increased above the level before the drug was given. But after more than 2 h under the influence of bicuculline (and even with an additional intravenous application; 0.4 mg kg^{-1}) statistically significant inhibitory side flanks to the tuning curve clearly persisted, with strong inhibition even for a stimulus perpendicular to the best orientation. This indicates the presence of some non-orientation-specific inhibition¹⁹, possibly not GABA mediated²⁰, which may operate in addition to the orientation-specific inhibition^{8,9} in some or all cells.

One cell was classified as pure direction selective, responding to movement of any stimulus in the optimal direction²¹. Its breadth of tuning for orientation increased by about 50%, and so did its peak response. This was, in fact, the only cell found showing an almost exact 'iceberg' effect, with constant slopes to the tuning curve before and after the drug, perhaps because of the removal of nonorientation-specific inhibition.

One hypercomplex cell has also been tested (in cortical layer VI: 10^{-4}M bicuculline). Before the application of bicuculline, a long bar (16°) caused slight inhibition of

Table 1 Actions of bicuculline on orientation tuning curves

Method of application	Cell type	Cortical layer	Change in breadth of tuning (%)	Change in peak response (%)	Ratio of changes (peak: breadth)	Change in spontaneous activity	Notes
Intravenous 0.2 mg kg^{-1}	Simple		(-15)	(+10)		(+)	
			(-5)	(-17)		+	
	Complex	IV	(+3)	-31		-	
		VI	+22	-27		(-)	
		VI	(-1)	+40		+	
Topical 10^{-4} or 10^{-5}M	Simple	V	(+12)	+31	2.6	(+)	*
		III	+31	+151	4.9	+	† (Fig. 1 <i>b</i>)
		II	+36	(-18)		(+)	†‡
	Complex	VI	+75	+23	0.3	(+)	*
		V	+20	+88	4.4	(-)	‡
		III	(-3)	-36		+	
		VI	+26	+100	3.8	(-)	(Fig. 1 <i>c</i>)
		VI	-17	-40		-	
	Pure direction selective	V	+49	+51	1.0	(+)	†

Data were collected starting 1–5 min and finishing 13–29 min after intravenous injection, and 5–20 min to 35–60 min after topical application. All cells studied topically were within 1.6 mm of the surface. (One complex cell was studied which was 4 mm deep, beyond the probable range of drug diffusion; no effect of bicuculline was seen, and the cell has been excluded from these results.) From tuning curves like those in Fig. 1*b* and *c* the breadth of tuning (average half width¹⁴) and the peak response (evoked impulses at the optimal orientation) were measured before and during the application. Changes in these parameters are expressed as % increases or decreases. Values in parentheses failed to meet the criteria of significance ($>15\%$ for breadth of tuning, $>20\%$ for peak response) established from control measurements of the inherent variability of these parameters without the application of the drug¹⁴. To test the prediction that the peak response should show a greater % increase than the breadth of tuning, the ratio of the two is shown for cells showing a positive change in both: ratios greater than 1.0 support the hypothesis. Changes in spontaneous activity are shown simply as an increase or a decrease (+ or -), those in parentheses being nonsignificant ($P > 0.01$). Not all cells were tested for changes in field size. Both cells examined for reduced direction selectivity (one simple, one complex) showed the effect.

* 10^{-5}M bicuculline (all others 10^{-4}M).

† Receptive field size increased.

‡ Direction selectivity reduced.

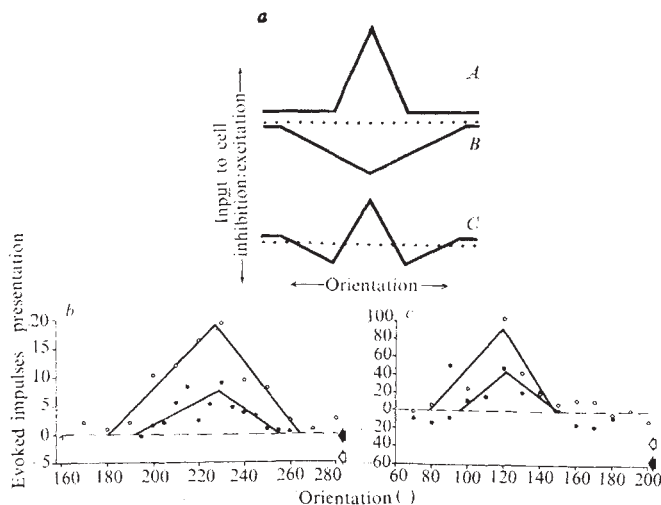


Fig. 1 *a* Diagrammatic illustration of the theory described in the text, whereby a broadly tuned excitatory input, *A*, and a more broadly tuned inhibitory input, *B*, summate (perhaps linearly, as shown here) to give the final orientational tuning curve *C*. Bicuculline should convert the cell's tuning curve from type *C* to type *A*. Normally, in cortical cells, spontaneous discharge is too low for inhibitory 'side flanks' to be seen clearly (the dotted lines represent zero firing level for the cell). Alternatively, inhibitory effects might be nonlinear, perhaps presynaptic, affecting the gain of the excitatory mechanisms? *b*, Effects of bicuculline on a simple cell, D20R4 (10^{-4} M bicuculline, 15–64 min after application). *c*, Effects of bicuculline on a complex cell, D18L6 (10^{-4} M, 5–50 min after application). ●, Responses before application; ○, responses after application. The spontaneous activity was counted over the same gating period used to measure the responses. For clarity, the responses are displayed on the ordinate as the extra impulses generated by the stimulus relative to these spontaneous levels. Inhibition appears as negative values, that is reductions of the spontaneous discharge. Zero firing levels before (◀) and after (◁) the administration of the drug are shown.

spontaneous activity, but with bicuculline, a long bar produced a clear response, 24% ($P < 0.05$) of that elicited by a short bar (1.5°). Two and a half to three hours after removal of the drug, long bars again inhibited the cell slightly.

There are numerous difficulties in interpreting these results quantitatively: one is the general problem of assessing the significance of experimental changes in the characteristics of neurons that exhibit inherent variability in responsiveness^{10,14,22} and a second is the uncertain nature of diffusional barriers, including distance, when a drug is applied topically. Finally, bicuculline itself occasionally has only weak or even anomalous actions as a GABA antagonist^{23,24}. In spite of these problems, however, all but one of the neurones in this sample showed some if not all of the predicted effects of bicuculline. These results, together with Sillito's very recent report of the actions of iontophoretically-applied bicuculline²⁴, warrant further investigations into the functions of GABA in the visual cortex.

We thank E. A. Tobin for her collaboration during a visit to Cambridge in 1971. Drs J. S. Kelly and J. G. Robson gave useful discussion and R. D. Loewenbein and J. S. Dormer gave technical assistance. D.R. is an MRC scholar; equipment was provided by the H. E. Durham Fund and an MRC grant to C.B.

DAVID ROSE
COLIN BLAKEMORE

The Physiological Laboratory,
Cambridge CB2 3EG, U.K.

Received January 14; revised February 21, 1974.

- ¹ Pettigrew, J. D., and Daniels, J. D., *Science*, **182**, 81–83 (1973).
- ² Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222–1224 (1970).
- ³ Krnjević, K., *Nature*, **228**, 119–124 (1970).

- ⁴ Hubel, D. H., and Wiesel, T. N., *J. Physiol. Lond.*, **160**, 106–154 (1962).
- ⁵ Hubel, D. H., and Wiesel, T. N., *J. Physiol. Lond.*, **195**, 215–243 (1968).
- ⁶ Andrews, D. P., *Vision Res.*, **7**, 975–997 (1967).
- ⁷ Carpenter, R. H. S., and Blakemore, C., *Expl Brain Res.*, **18**, 287–303 (1973).
- ⁸ Benevento, L. A., Creutzfeldt, O. D., and Kuhnt, U., *Nature new Biol.*, **238**, 124–126 (1972).
- ⁹ Blakemore, C., and Tobin, E. A., *Expl Brain Res.*, **15**, 439–440 (1972).
- ¹⁰ Bishop, P. O., Coombs, J. S., and Henry, G. H., *J. Physiol. Lond.*, **219**, 659–687 (1971).
- ¹¹ Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **28**, 229–289 (1965).
- ¹² Bishop, P. O., in *Handbook of Sensory Physiology* (edit. by Jung, R.) **VIIA**, 225–305 (Springer, Berlin, 1973).
- ¹³ Blakemore, C., Donaghy, M. J., Maffei, L., Movshon, J. A., Rose, D., and Van Sluyters, R. C., *J. Physiol. Lond.*, **237**, 39–41 (1974).
- ¹⁴ Rose, D., and Blakemore, C., *Expl Brain Res.*, **20**, 1–17 (1974).
- ¹⁵ Straschill, M., and Perwein, J., *Pflügers Arch. ges. Physiol.*, **312**, 45–54 (1969).
- ¹⁶ Phillis, J. W., *Int. Rev. Neurobiol.*, **14**, 1–48 (1971).
- ¹⁷ Tebécis, A. K., Hösl, L., and Haas, H. L., *Experientia*, **27**, 548 (1971).
- ¹⁸ Precht, W., Baker, R., and Okada, Y., *Expl Brain Res.*, **18**, 415–428 (1973).
- ¹⁹ Bishop, P. O., Coombs, J. S., and Henry, G. H., *J. Physiol. Lond.*, **231**, 31–60 (1973).
- ²⁰ Phillis, J. W., and York, D. H., *Brain Res.*, **5**, 517–520 (1967).
- ²¹ Blakemore, C., and Van Sluyters, R. C., *J. Physiol. Lond.*, **237**, 195–216 (1974).
- ²² Henry, G. H., Bishop, P. O., Tupper, R. M., and Dreher, B., *Vision Res.*, **13**, 1771–1779 (1973).
- ²³ Straughan, D. W., Neal, M. J., Simmonds, M. A., Collins, G. G. S., and Hill, R. G., *Nature*, **233**, 352–354 (1971).
- ²⁴ Sillito, A. M., *J. Physiol. Lond.* (in the press).

Constitutivity of the HCG-receptor protein in the testis of rat and man

In a series of embryological experiments on rabbits and rats, Jost *et al.*¹ have shown that the manifestation of the male or female phenotype is strictly dependent on the presence or absence, respectively, of testosterone. The Müllerian inhibitor formed by the seminiferous tubules² only seems to be responsible for the retrogression of the Müllerian ducts. Thus, testosterone synthesised in foetal testes³ is the chief organiser of masculine differentiation; a female phenotype results from the absence of testosterone. Accordingly, Ohno⁴ defined maleness as the induced and femaleness as the noninduced state of genital differentiation. The androgenic effects on the development of the genital structures occur during successive critical phases. For instance, the stabilisation of the Wolffian ducts in the rat occurs between days 17 and 18 (ref. 1) while the prostate is imposed on day 21 of foetal life. The imprinting of the rat hypothalamus into the male direction, responsible for the masculine secretory pattern of pituitary gonadotrophins and behaviour pattern, is established during the first two days of postnatal life⁵. Castration, hypophysectomy or injections of cyproterone acetate (a highly potent anti-androgen) before these critical stages prevent the differentiation processes but by later treatment the development is no longer affected. Similar developmental patterns may be applicable to man except for hypothalamus differentiation which is fixed as early as in the fifth month of intrauterine life⁶. Full maturity of the genital structures and particularly the onset of sperm production, are both dependent on testicular androgens, so occur rather late during the postnatal life of all mammalian species.

Testicular androgens are synthesised by stimulus of the gonadotrophic hormones, luteinising hormone (LH) or human chorionic gonadotrophin (HCG). Recent studies have shown that the gonadotrophic effect is mediated by a

Table 1 Uptake of ^{125}I -HCG by rat testicular tissue taken from pre and postnatal rats. The spleens of the prenatal animals were used as controls.

	c.p.m. bound without unlabelled HCG	c.p.m. bound plus unlabelled HCG (30 μg)	Specific uptake of ^{125}I -HCG ($\text{g} \times 10^{-10}$) per 30 min per mg protein
Rat testis prenatal	$3,478 \pm 319^*$	731 ± 183	1.16
Rat spleen prenatal	710 ± 41	725 ± 120	0
Rat testis day 5	$1,966 \pm 9.9$	627 ± 68	0.35
Rat testis day 10			0.85†
Rat testis day 20			1.76†
Rat testis adult			3.18†

Binding studies, purification and iodination of HCG were performed as described earlier⁹. All assays were performed in triplicate. The amount of specifically bound HCG was determined as the difference between the c.p.m. bound in samples with ^{125}I -HCG alone and in samples containing an excess of unlabelled HCG.

* Standard error of the mean.

† See ref. 9.

specific HCG-receptor in the testicular Leydig cells; the stimulation of testosterone synthesis by HCG in Leydig cells is preceded by the binding of the polypeptide hormone to its specific receptor⁷. The presence of the specific HCG receptor in the developing Leydig cell should therefore be a prerequisite for testosterone synthesis and also male organogenesis in the foetus. As testosterone synthesis is required during both pre and postnatal development, it might be assumed that the HCG receptor is present throughout these periods, appearing with the first formation of Leydig cells in the primordial testis. These cells are first seen in the rat on day 14 (ref. 1) and in man around the 60th

The important point is, however, that the binding capacity for HCG of the Leydig cell at each developmental stage in rat and man is sufficient to evoke a maximum response; Catt and Dufau⁷ have shown that less than 1% of the HCG-receptor sites must be occupied for maximum testosterone synthesis. We conclude from our results that one of the first events occurring in the differentiating Leydig cell is the 'switching on' of the receptor synthesis so that the Leydig cells can start testosterone synthesis. The receptors become functional to guarantee that androgenic hormone synthesis is sufficient for the stepwise maturation of the androgen-dependent genital structures. Defects in the gonadotrophin

Table 2 Uptake of ^{125}I -HCG by human testicular tissue at different developmental stages.

	26th foetal week	Neonate (day 4)	10 yr	46 yr	75 yr
Specific uptake of ^{125}I -HCG ($\text{g} \times 10^{-10}$) per 30 min per mg protein.	0.451 ± 0.05	0.208 ± 0.017	1.800 ± 0.12	0.293 ± 0.03	0.217 ± 0.019

For experimental procedure see text and legend of Table 1.

day of foetal life⁸. Here we report experiments to test this hypothesis by measuring specific binding of ^{125}I -HCG by foetal and newborn rats and human testicular tissue.

The procedure used was similar to that described earlier⁹. For the study of prenatal animals at a definite developmental stage, mating was determined by examining the presence of copulation plugs in the female rats. On the 20th day of pregnancy the animals were killed and the foetal testes removed. The testes of between 60 and 70 animals were taken together and homogenised in cold Tris-HCl buffer (0.1 M, pH 7.4) containing MgCl_2 (0.005 M) and sucrose (0.1 M); when 5-d-old rats were used, the testes of 15 animals were pooled in each assay. In the 10, 46 and 75-yr-old humans individual tests were removed 3–5 h after accidental death; the neonate died from pneumonia. Portions (1 ml) of the homogenate were incubated with 7 ng ^{125}I -HCG alone (about 5×10^4 c.p.m.), or with the addition of 30 μg unlabelled HCG respectively at 32° C for 30 min (Table 1). After incubation unbound radioactivity was separated by centrifugation.

The results clearly demonstrate the presence of a specific HCG receptor in the pre and postnatal testis of the rat and man (Tables 1 and 2). In contrast to the rat, specific HCG-binding by testicular tissue in man does not vary much with age; only the 10-yr-old boy showed a relative high binding capacity. It is not yet known whether there is any correlation between high binding capacity and the onset of puberty. In rats the fluctuation in binding might be due to the varying amount of Leydig cells present during the respective developmental stages¹⁰. There is evidence¹¹ that the pattern of change in Leydig cell numbers may be similar in rat and human testicular development. Further studies are in progress to discover any correlations between hormone binding and human testicular developmental stages.

receptor may result not only in infertility of the adult male but also in defects in the differentiation of male primary and secondary sexual organs. Such defects may only be apparent in the homozygous state, heterozygosity may not reveal deviations, as only a very small quantity of HCG receptors must be occupied to evoke biological response of the Leydig cells.

Constitutivity has already been supposed for the androgen receptor in the rat ventral prostate¹² and for the oestrogen receptor in the rat uterus¹³. To our knowledge, the results presented here represent the first example of constitutivity of a hormone receptor protein in mammals.

This work was supported by the Deutsche Forschungsgemeinschaft. Crude HCG was a gift from Shering AG, Berlin and Organon, Oss.

JOACHIM FROWEIN
WOLFGANG ENGEL

*Institut für Humangenetik und
Anthropologie der Universität,
78 Freiburg i Br.
Albertstrasse 11,
West Germany*

Received December 27, 1973; revised February 18, 1974.

¹ Jost, A., Vigier, B., Prépín, J., and Perchellet, J. P., *Rec. Progr. Horm. Res.*, **29**, 1 (1973).

² Jossé, N., *Endocrinology*, **93**, 829 (1973).

³ Noumoura, T., Weisz, J., and Lloyd, C. W., *Endocrinology*, **78**, 245 (1966).

⁴ Ohno, S., *Hereditas*, **69**, 107 (1971).

⁵ Harris, G. W., *Endocrinology*, **75**, 627 (1964).

⁶ Dörner, G., *Sexualhormonabhängige Gehirndifferenzierung und Sexualität* (Springer-Verlag, Wien-New York, 1972).

⁷ Catt, K. J., Dufau, M. L., *Nature new Biol.*, **244**, 219 (1973).

⁸ Reyes, F. I., Winter, J. S. D., and Faiman, C., *J. clin. Endocr. Metab.*, **37**, 74 (1973).

- ⁸ Frowein, J., Engel, W., Weise, H. Ch., *Nature new Biol.*, **246**, 148 (1973).
¹⁰ Lording, D. W., and De Kretser, D. M., *J. Reprod. Fert.*, **29**, 261 (1972).
¹¹ Mancini, R. E., Vilar, O., Lavieri, J. C., Andrada, J. A., and Heinrich, J. J., *Am. J. Anat.*, **122**, 203 (1963).
¹² Sullivan, J. V., and Strott, C. A., *J. biol. Chem.*, **248**, 3202 (1973).
¹³ Luck, D. N., Gschwendt, M., Hamilton, T. H., *Nature new Biol.*, **245**, 24 (1973).

Fibril structure of collagen in egg capsule of dogfish

In spite of a wealth of information relating to the composition and structure of mesenchymal collagens, relatively little is known about the so-called 'secreted' collagens of epithelial origin which are much more limited in distribution. The materials of the selachian egg capsule has been classified as such a collagen though this claim is based on a poorly oriented high angle X-ray diffraction pattern obtained from skate egg capsules¹ and on the colorimetric detection of hydroxyproline in hydrolysates of the same material². Partial amino acid analysis of this material² indicated a composition markedly different from pure collagens. To date there has been no report of fibrous periodicity in selachian egg capsules.

Here, we provide evidence that the inner layers of the egg capsule wall of the selachian, *Scyliorhinus caniculus* are built up from fibrils which contain a collagen-like protein, probably in association with other protein(s). The unique and highly ordered structure of these fibrils is described.

Fresh egg capsules were obtained from the Scottish Marine Biological Association Laboratories. Electron microscopical examination of ultrathin sections of the egg capsule wall fixed in glutaraldehyde and osmium tetroxide and embedded in Epon showed that the inner layers of the capsule wall consist of laminae (approximately 0.4 μm thick)

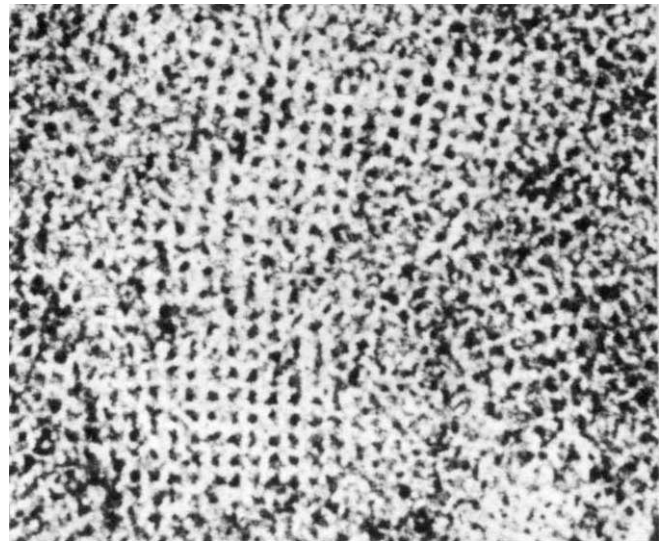


Fig. 2 Transverse section showing the intrafibrillar lattice (stained with uranyl acetate and lead citrate (X 335,000)).

which seem to be built up entirely from closely packed fibrils. In a single lamina, the long axes of the fibrils are parallel. Laminae appear stacked upon each other to give a cross ply construction such that there is an angle of approximately 45° between the long axes of the fibrils in adjacent laminae. The outermost layer of the capsule shows a different construction and is less transparent and more pigmented than the inner layers. In ultrathin sections it seems to consist of closely-packed elliptical granules (0.2–0.5 μm in diameter). Small quantities of fibrils similar to those of the inner layers are found in the interstices between the granules. Amino acid analyses of the outermost and the inner layers (Table 1) were obtained using a Locarte analyser. The outermost layer differed from the inner layers and showed a high tyrosine content (18 residues/100 residues).

The inner layers showed an amino acid composition clearly different from pure mammalian collagen (Table 1). Hot trichloroacetic acid³, however, extracted protein with a composition showing a resemblance to pure mammalian collagen and an even closer resemblance to the 'secreted' collagen of the byssus threads of *Mytilus edulis* (Table 1).

A moderately well oriented high angle X-ray diffraction pattern was obtained after slowly stretching the marginal band of the capsule to an extension of 50–70%. This pattern had the following features in common with collagen⁵: a strong meridional arc at 2.9 Å, and a weaker one at 4 Å. A broad diffuse equatorial arc at 4.5 Å, a faint diffuse equatorial spot at 6 Å and a strong one at 12 Å which on wetting became a streak with a maximum intensity at about 15 Å. Strips of the inner layers showed a sharp thermal transition (mean half shortening temperature 78° C), shrinking by about 30–40% of their resting length.

Taken together, this evidence indicates that the fibrils which comprise the inner layer of the capsule contain a protein with the configuration of a collagen together with other protein(s) not in this configuration. In this respect the fibrils appear to resemble those of elastoidin⁶.

Fibrils of the inner layers of the capsule seen in ultrathin sections stained with uranyl acetate and lead citrate have a mean diameter of 1,300 Å and show an axial, centrosymmetrical banding pattern with a mean repeat period of 370 Å (Fig. 1a). This banding pattern is built up from alternating narrow bands (A; 70 Å wide) and broad bands (B; 120 Å wide), both of low density, separated by bands (C; 90 Å wide) of high density. The C bands can be resolved into two dense sub-bands. Fibrils dispersed from the inner layers by homogenisation and sonication show, on negative staining

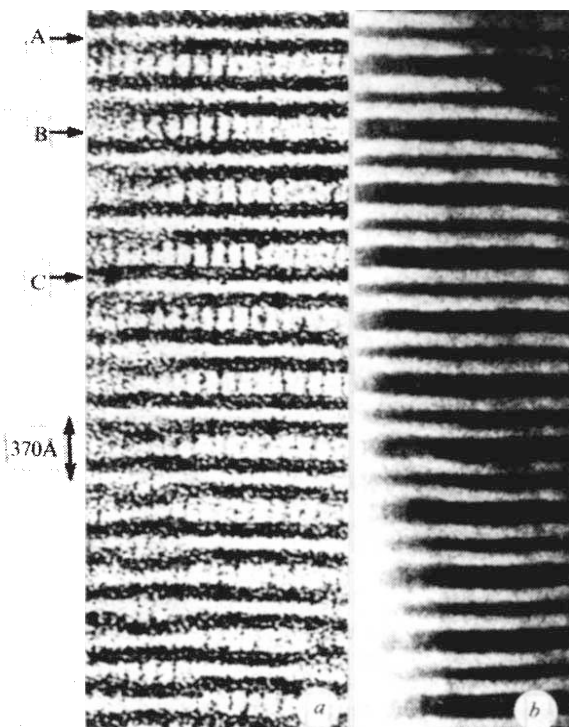


Fig. 1a, Axial banding pattern. Positively stained ultrathin sections. b, Negatively stained dispersed fibrils.

Table 1 Amino acid composition in residues per 100 amino acid residues. Corrected for hydrolytic losses.

	Inner layer	Outer layer	TCA extract of inner layer trace	TCA extract of byssus threads*	Bovine skin collagen†
cysteic acid	1.2	1.2		—	—
OH Pro	0.9	0.4	4.4	7.1	9.2
Asp	11.0	13.3	6.6	6.7	4.8
Thr	5.6	3.6	4.6	3.4	1.7
Ser	6.2	7.0	4.8	4.2	3.8
Glu	6.7	7.0	6.5	8.9	7.2
Pro	7.9	7.7	9.9	8.3	12.9
Gly	16.0	16.5	30.0	33.7	33.4
Ala	6.5	3.6	8.0	8.4	10.5
Val	5.2	2.1	3.2	3.3	1.9
Met	trace	0.6	2.7	0.2	0.7
Ileu	3.3	1.5	1.8	1.7	1.1
Leu	3.5	1.5	2.1	2.2	2.5
Tyr	8.0	18.1	2.4	0.8	0.5
Phe	3.2	2.5	1.3	1.0	1.3
His	3.1	5.5	1.5	1.1	0.5
OH Lys	—	—	0.1	0.2	0.7
Lys	7.1	4.7	3.5	7.1	2.5
Arg	4.5	2.9	6.6	4.5	4.8

* 'Secreted' collagen of the byssus threads of *Mytilus edulis* (D. P. K., and S. O. Anderson, unpublished).

† Extracted as gelatin⁴.

with PTA or ammonium molybdate at pH 6.4, a banding pattern closely similar to that seen in positively stained ultrathin sections but in reversed contrast (Fig. 1b).

Two observations indicate that the transverse arrangement of molecules within the B bands is highly ordered. First, where the fibrils are sectioned transversely, a highly ordered array of dense dots is seen (Fig. 2). These lie in a nearly square, rhombic lattice (lattice angle 93.5°; mean length of equilaterals 80 Å). Second, in longitudinal sections where a fibril is appropriately orientated about its long axis, a pattern of evenly spaced longitudinal filamentous densities is seen traversing the B band (Fig. 1a). These show spacings of either approximately 80 Å or approximately 60 Å and

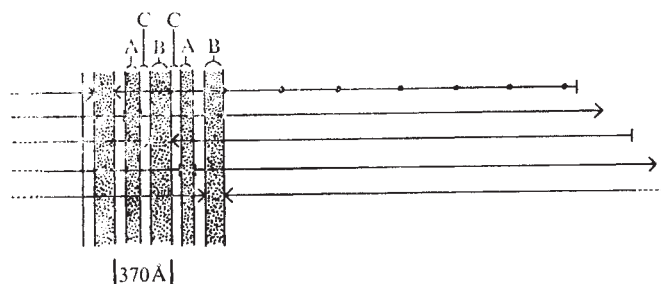


Fig. 3 Diagram illustrating the proposed longitudinal arrangement of molecules in the fibrils. Negative stain is thought to penetrate the gaps between the head ends and the tail ends. Arrowheads and bars indicate only the opposite ends of the molecule and do not imply which is the amino end.

may correspond respectively to images of the rhombic lattice viewed in the plane of the equilaterals or in the plane of the diagonals.

Although we have been unable to devise a model to account for all the features in our micrographs, the centrosymmetrical nature of the axial banding pattern and the alternation of narrow and broad bands into which negative stain can penetrate indicate that fibrous molecules are arranged longitudinally in a head-to-head, tail-to-tail fashion with gaps between the head ends of different size to the gaps between the tail ends (Fig. 3).

Although we have, so far, been unable to determine the molecular length of the collagen in this system, if it were close to that of a mammalian collagen (2,990 Å, ref. 7), the molecules would need to be staggered by about $\frac{1}{8}$ of the molecular length to produce the repeat of 370 Å (Fig. 3). Eight collagen molecules staggered in this way and packed together transversely in some form of nearly square array

could account for the axial periodicity and could provide the building unit for the nearly square lattice seen in transverse sections of the fibrils.

The collagen-containing fibrils of the inner layers of the dogfish egg capsule show a unique fine structure indicating a highly ordered longitudinal and transverse arrangement of molecules. Our demonstration by electron microscopy of a nearly square unit cell (sides 80 Å) in transverse sections of these fibrils is particularly interesting in the light of the evidence from low angle X-ray diffraction for a similar lattice (sides 77 Å) (ref. 8) in the collagen fibrils of rat tail tendon.

We thank the Science Research Council for support and K. Oates and A. Watson for assistance.

D. P. KNIGHT
S. HUNT

Department of Biological Sciences,
University of Lancaster,
Bailrigg, Lancaster, UK

Received January 4; revised February 19, 1974.

- 1 Bear, R. S., *Adv. Protein Chem.*, **7**, 69 (1952).
- 2 Gross, J., Dumsha, B., and Glazer, N., *Biochim. biophys. Acta*, **30**, 293 (1958).
- 3 Fitch, S. M., Harkness, M. C. R., and Harkness, R. D., *Nature*, **176**, 163 (1955).
- 4 Bailey, A. J., *Comp. Biochem.*, **26B**, 297 (1968).
- 5 Ramachandran, G. N., Sasisekharan, V., and Thathachari, Y. T., in *Collagen* (edit. by Ramanathan, N.) (J. Wiley, New York, 1960).
- 6 Kimura, S., and Kubota, M., *Bull. Jap. soc. scient. Fish.*, **34**, 535 (1968).
- 7 Piez, K. A., Miller, E. J., Lane, J. M., and Butler, W. T., in *Chemistry and Molecular Biology of the Intercellular Matrix*, **1** (edit. by Balazs, E. A.) (Academic Press, New York and London, 1970).
- 8 Miller, A., and Parry, D. A. D., *J. molec. Biol.*, **75**, 441 (1973).

Release of cyclic AMP from rat superior cervical ganglia after stimulation of synthesis *in vitro*

THE formation of adenosine 3',5'-monophosphate (cyclic AMP) from adenosine 5'-triphosphate (ATP) in central nervous tissues seems to be controlled by catecholaminergic and histaminergic mechanisms¹. Also in a peripheral autonomic ganglion, the intact superior cervical ganglion (SCG), accumulation of cyclic AMP was observed in response to electric stimulation² and to β -adrenergic agents³, and histamine^{4,5}.

Cyclic AMP is excreted in large amounts in the urine⁶, and

Table 1 Accumulation of cyclic AMP in SCG and release of cyclic AMP to the medium during incubation in the presence of adrenaline; acetylcholine; adrenaline after preincubation with practolol; or adrenaline and acetylcholine together

	N	Control	N	Adrenaline 10 ⁻⁴ M for 15 min	N	Acetylcholine 10 ⁻³ M for 15 min	N	Adrenaline plus practolol	N	Adrenaline plus acetylcholine
Cyclic AMP in ganglia (pmol $\pm$ SD per ganglion)	3	4.2 $\pm$ 0.2	3	24.9 $\pm$ 0.05*	3	4.8 $\pm$ 0.02	3	5.2 $\pm$ 0.8	3	24.9 $\pm$ 1.2*
Cyclic AMP in medium (pmol $\pm$ SD per ganglion)	3	0.6 $\pm$ 0.1	3	1.2 $\pm$ 0.2†	3	0.5 $\pm$ 0.06	3	0.6 $\pm$ 0.1	3	1.2 $\pm$ 0.1†

*P < 0.01 Student's *t*-test. †P < 0.05 Student's *t*-test.

marked changes in the excretion rate correlate with mood changes in manic depressed patients and with response to central active drugs⁷⁻⁹. It has therefore been suspected and is supported by indirect evidence⁸ that the central nervous system may be one important source of cyclic AMP in body fluids.

We have reported experimental data showing a relationship between the rate of accumulation of cyclic AMP in the cerebrospinal fluid and the amount of catecholamine synthesis in the brain¹⁰. These findings prompted us to study more directly the release of cyclic AMP from nervous tissues when the synthesis of the nucleotide was stimulated by biogenic amines *in vitro*.

Male Sprague Dawley rats, weighing 200 to 300 g, were killed by cervical dislocation. The superior cervical ganglia (SCG) were rapidly removed, desheathed, and kept on ice until used for incubations. The ganglia were incubated in groups of two to four in 1 ml of Krebs-Henseleit solution pH 7.4 containing 200 mg ml⁻¹ of ascorbic acid at 37° C under an atmosphere of 95% O₂ and 5% CO₂ with or without the drugs added. Blocking agents were tested by a preincubation period of 20 min. At the end of the experiments the ganglia were homogenised and chromatographed on anion exchange columns as described³. Total cyclic AMP was measured in column eluates of ganglia and in incubation media according to Gilman¹¹. The formation of ¹⁴C-cyclic AMP was measured after preincubation of the ganglia with ¹⁴C-adrenaline for 1 h as described for brain slices¹². Labelled cyclic AMP was determined by thin layer chromatography on silica-gel sheets¹³. Cyclic AMP phosphodiesterase activity was determined at micromolar substrate concentration according to Thompson and Appleman¹⁴, lactate dehydrogenase activity by the method of Wroblewski and LaDue¹⁵, and protein by the method of Lowry *et al.*¹⁶.

The levels of cyclic AMP in freshly dissected SCG of rats ranged from 1.9 to 4.2 pmol per ganglion, corresponding to 1.6 to 2.3 pmol per mg fresh tissue. If SCG were incubated for 5–10 min in the absence of drugs, only small amounts of cyclic AMP were detectable in the medium (Fig. 1). Adrenaline (10⁻⁴ M), noradrenaline (10⁻³ M), isoprenaline (10⁻⁵ M), and histamine (10⁻³ M) rapidly increased the levels of cyclic AMP in SCG tissue in a dose-related manner. The time course of the accumulation of cyclic AMP on incubation with adrenaline is shown in Fig. 1.

Elevated cyclic AMP concentrations were observed in the medium after 3 min incubation of the ganglia with adrenaline (10⁻⁴ M) or histamine (10⁻³ M). The rise of the cyclic AMP concentration seemed to continue after the peak accumulation was reached in the ganglia but the medium levels decreased

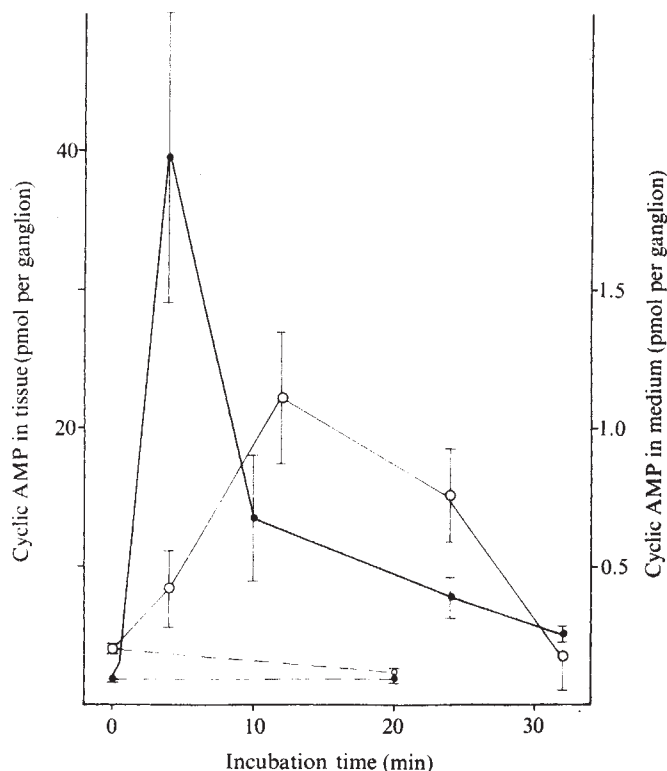


Fig. 1 Time course of the accumulation of cyclic AMP in superior cervical ganglia and in the incubation medium during incubation with L-adrenaline (10⁻⁴ M). Cyclic AMP was determined according to Gilman¹¹. ●, Cyclic AMP content of ganglia; ○, cyclic AMP appearing in the medium; ---, time course in control incubations without the amine. Each point represents the mean of four experiments. Vertical bars show double standard errors of the mean.

again after 12 to 15 min of stimulation (Fig. 1). Acetylcholine (10⁻³ M) and carbamylcholine (10⁻⁹–10⁻³ M) did not alter the cyclic AMP concentration in the SCG and in the incubation medium. Moreover, acetylcholine did not influence the accumulation and release of cyclic AMP induced by adrenaline. Preincubation with propranolol or practolol, two β -adrenergic blocking agents, completely blocked the effects of adrenaline on cyclic AMP in SCG and the medium (Table 1).

The assay for cyclic nucleotide phosphodiesterase activity in the incubation medium revealed the appearance of protein

Table 2 Medium content of total protein, cyclic nucleotide phosphodiesterase activity and lactate dehydrogenase activity after incubation of superior cervical ganglia for various times

Treatment	Protein (μ g per ganglion)	Cyclic nucleotide phosphodiesterase (pmol cyclic AMP hydrolysed per mg protein per min)	Lactate dehydrogenase (mU per ml per mg protein per min)
Control (10 min)	11.5	13.5	7.4
Histamine 10 ⁻³ M (10 min)		6.7	5.3
Control (40 min)	9.6	12.0	24.3
Histamine 10 ⁻³ M (40 min)		4.8	18.7

and enzyme activity in the absence of stimulating drugs (Table 2). While the activity of lactate dehydrogenase which serves as a cytoplasmic marker enzyme increased in the medium during incubation with three- to four-fold increases from 10–60 min, bulk release of protein as well of phosphodiesterase activity occurred mainly during the initial period of incubation with in general no further increases in concentration and specific activity after 10 min of incubation. When ganglia were incubated with adrenaline or histamine the phosphodiesterase activity was not increased in the medium but slightly decreased while the total release of proteins from the ganglia into the medium was unchanged. This behaviour may indicate that cyclic nucleotide phosphodiesterase in an activated state is less prone to loss from intracellular compartments than in an inactive form.

After preincubation of SCG with ^{14}C -adenine for 60 min, less than 1% of the total radioactivity was found to correspond to ^{14}C -cyclic AMP. Of the total ^{14}C -cyclic AMP formed 35–45% appeared in the medium if measured after 15 min incubation in the absence of drugs. The addition of adrenaline or histamine strongly stimulated the rate of conversion to ^{14}C -cyclic AMP. Of the total radioactivity 6 and 5% was associated with cyclic AMP after incubation with adrenaline (10^{-4} M) and histamine (10^{-3} M) respectively for 15 min. The ratio of labelled cyclic AMP in the medium to ^{14}C -cyclic AMP in the ganglia remained essentially unchanged after stimulation as compared to the resting condition. This behaviour indicates that the preincubation with ^{14}C -adenine rather selectively labels a separate ATP precursor pool for cyclic AMP, as was observed earlier^{12,17} and, in addition, suggests a membrane associated localisation of the precursor pool leading to preferential release of labelled product after the stimulation of adenylate cyclase by biogenic amines.

Our experiments have shown that from a peripheral sympathetic ganglion of the rat cyclic AMP is released following the stimulation of its intracellular formation by biogenic amines such as adrenaline and histamine *in vitro*. Acetylcholine neither affects the rate of synthesis nor the release of cyclic AMP. Whether under physiological conditions the release or overflow of cyclic AMP after stimulation by neurohormones serves to remove, and thus inactivate, excessive cyclic AMP unbound to protein kinases, or whether released cyclic AMP has some function of its own, is at present unclear. Evidence in favour of a release of cyclic AMP from central nervous tissues *in vivo* has also been obtained in humans¹⁸.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

H. CRAMER
T. LINDL

Department of Neurology,
University of Freiburg
Hansastraße 9, 7800 Freiburg, West Germany

Received November 1, 1973; revised March 11, 1974.

- ¹ Kakiuchi, S., and Rall, T. W., *Molec. Pharmacol.*, **4**, 379 (1968).
- ² McAfee, D. A., Schorderet, M., and Greengard, P., *Science*, **171**, 1156 (1971).
- ³ Cramer, H., Johnson, D. G., Hanbauer, I., Silberstein, S. D., and Kopin, I. J., *Brain Res.*, **53**, 97 (1973).
- ⁴ Lindl, T., and Cramer, H., *IRCS* (73–8) 3–5–12.
- ⁵ Lindl, T., and Cramer, H., *Biochim. biophys. Acta*, **343**, 182 (1974).
- ⁶ Chase, L. R., Aurbach, G. D., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 518 (1967).
- ⁷ Paul, M. I., Ditzion, B. R., Janowsky, D. S., *Lancet*, **i**, 88 (1970).
- ⁸ Abdulla, Y. H., and Hamadah, K., *Lancet*, **i**, 378 (1970).
- ⁹ Paul, M. I., Cramer, H., and Goodwin, F. K., *Arch. gen. Psychiat.*, **24**, 327 (1971).
- ¹⁰ Cramer, H., and Lindl, T., *Psychopharmacologia*, **26**, supplement, 49, (1972).
- ¹¹ Gilman, A., *Proc. natn. Acad. Sci., U.S.A.*, **67**, 305 (1970).
- ¹² Shimizu, H., Daly, J. W., and Creveling, C. R., *J. Neurochem.*, **16**, 1609 (1969).
- ¹³ Dighe, P. K., Pahuja, D. N., and Sha, D. H., *J. Chromatogr.*, **40**, 449 (1969).
- ¹⁴ Thompson, W. J., and Appleman, M. M., *Biochemistry*, **10**, 311 (1971).

- ¹⁵ Wroblewski, F., and La Due, J. S., *Proc. Soc. exp. Biol. Med.*, **90**, 210 (1955).
- ¹⁶ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- ¹⁷ Schultz, J., and Daly, J. W., *J. biol. Chem.*, **248**, 843 (1973).
- ¹⁸ Cramer, H., Ng, N.K.Y., and Chase, T. N., *J. Neurochem.*, **19**, 1601 (1972).

Origins of metal ions in biology

A FERREDOXIN may have been one of the earliest proteins formed. The evidence for this assumption was recently presented by Hall *et al.*¹ based on the primary structures of several ferredoxins. Here, further evidence is reported for the early evolution of ferredoxins, and the origins of ferredoxins and other metalloproteins are discussed from an inorganic point of view based on thermodynamic data.

The atmosphere predominating during the time of chemical evolution and early life is generally assumed to have been a reducing one, characterised by a moderate pressure of methane (0.01–0.001 atm) and a very low oxygen pressure². At such a reducing condition, thermodynamic data for the system, air–sea–sediment³, indicate that at equilibrium the redox potentials in the seas were as low as -325 mV ($p^E = -5.5$): inorganic sulphur would be in the form of the iron sulphide, $\text{FeS}_2(\text{s})$ (pyrite), and perhaps partly as $\text{Fe}_{0.86}\text{S}(\text{s})$ (pyrrhotite); the major excess of iron would be in the form of $\text{Fe}_3\text{O}_4(\text{s})$ and a minor amount dissolved in the form of Fe^{2+} (≤ 0.2 mM).

Crystals of $\text{FeS}_2(\text{s})$ and $\text{Fe}_{0.86}\text{S}(\text{s})$ are well-known semiconductors having the capacity to transfer electrons. This leads to the following hypothesis: inorganic electron carriers in the form of $\text{FeS}_2(\text{s})$ and $\text{Fe}_{0.86}\text{S}(\text{s})$ were present prebiotically in the area where the first proteins evolved. When polypeptides containing cysteine residues formed, they reacted spontaneously with these iron sulphides forming bonds similar to those present in ferredoxins (compare refs 4–6). Also, iron in the form of Fe^{2+} would react forming iron proteins similar to rubredoxin⁵. Thus, very early, at least two different kinds of iron-sulphur proteins most likely formed, and these proteins probably developed independently into two different classes of electron carriers. It should be noted that during these reducing conditions low-valent molybden could enter solid solutions of iron sulphide (molybdenite, $\text{MoS}_2(\text{s})$, would be the prevailing molybden compound), and the sulphur-rich Mo-Fe proteins⁷ might have evolved from such solid solutions (compare ref. 8).

The great abundance of iron in sea sediment supports the idea that the formation of iron-sulphur proteins was spontaneous and very common in early life processes. Also, the relative high concentration of dissolved iron (0.2 mM) would facilitate reactions with porphyrins, and thus the formation of haem proteins. Likewise, the Mn(II) concentration was much higher in the primordial seas³, perhaps as high as 50 mM. This may explain why manganese seems to be one of the most important elements in early redox processes; for instance, in the photosynthesis of the primitive blue-green algae⁹, and in the reactions of an early superoxy dismutase, apparently a manganese enzyme rather than a copper enzyme¹⁰.

During the early reducing conditions, copper ions were most likely trapped into extremely insoluble Cu(I) sulphides; they could not be released into aqueous and biological phases until the air–sea–sediment system became more oxidised by evolving oxygen. Then copper proteins developed, such as plastocyanin and cytochrome oxidase, which were required for further biological evolution. It can be estimated, using Sillén's data^{3,11}, that Cu(I) complexes of thiols and amines could form at redox potentials of about $+90$ and $+230$ mV, and free Cu^{2+} at about $+250$ mV. Thus, thermodynamic data strongly support an evolution of metalloproteins, beginning with iron and manganese proteins and continuing with copper proteins.

When oxygen successively oxidised the air-sea-sediment quite insoluble phases of iron and manganese formed³, FeOOH(s) and MnO₂(s). And at present, the concentrations of dissolved iron and manganese in sea water¹² are less than 10⁻⁷ M. Therefore, although copper was released by oxidation, iron and manganese became immobilised.

Also of particular evolutionary interest is the successive biological importance of weakly interacting metal ions; Mg²⁺ and Ca²⁺, for instance. Magnesium ions are important in reactions simulating prebiotic condensations¹³ and in early and fundamental cell reactions, such as protein biosynthesis, anaerobic energy production, and photosynthesis; calcium ions are of specific importance in processes characterising later and higher forms of life, such as nerve transmission, hormone secretion, and muscle contraction. One explanation for the early role of Mg²⁺ may be ascribed to its abundance; its high concentration in the oceans (54 mM) may have been even higher during the early reducing conditions, since on the primitive Earth NH₄⁺, Fe²⁺, and Mn²⁺ would partly replace Mg²⁺ in the silicate phases³.

R. ÖSTERBERG

Department of Medical Biochemistry,
University of Göteborg,
S-400 33 Göteborg,
Sweden

Received December 19, 1973; revised March 6, 1974.

- ¹ Hall, D. O., Cammack, R., and Rao, K. K., *Nature*, **233**, 136-138 (1971).
- ² Ponnampuram, C., *Q. Rev. Biophys.*, **4**, 77-106 (1971).
- ³ Sillén, L. G., *Arkiv Kemi*, **25**, 159-176 (1966).
- ⁴ Malkin, R., and Rabinowitz, J. C., *Biochem. biophys. Res. Commun.*, **23**, 822-827 (1966).
- ⁵ McCarthy, K., and Lovenberg, W., *J. biol. Chem.*, **243**, 6436-6441 (1968).
- ⁶ Sugiyama, Y., and Tanaka, H., *Biochem. biophys. Res. Commun.*, **46**, 335-340 (1972).
- ⁷ Hardy, R. W. F., Burns, R. C., and Parshall, G. W., *Bioinorganic Chemistry*, (edit. by Gould, R. F.) 219-247 (American Chemical Society, Washington, DC, 1971).
- ⁸ Schrauzer, G. N., and Schlesinger, G., *J. Am. chem. Soc.*, **92**, 1808-1809 (1970).
- ⁹ Olson, J. M., *Science*, **168**, 438-446 (1970).
- ¹⁰ Keele, B. B., Jr., McCord, J. M., and Fridowich, I., *J. biol. Chem.*, **245**, 6176-6181 (1970).
- ¹¹ Sillén, L. G., and Martell, A. E., *Stability Constants*, (Second ed.), (Chem. Soc. Spec. Publ. No. 17, 1964; Supplement No. 1, Chem. Soc. Spec. Publ. No. 25, 1971).
- ¹² Sillén, L. G., *Oceanography*, **0**, 549-581 (1959).
- ¹³ Lohrmann, R., and Orgel, L. E., *Nature*, **244**, 418-420 (1973).

Artificial induction of the sexual cycle of *Neurospora crassa*

CHEMICAL regulation of sexual development in fungi was suggested in the 1880s by Sachs and de Bary (see ref. 1). Burgeff² later demonstrated that the so-called + and - strains of *Mucor mucedo* if separated by a colloidal membrane, produced zygothecia that grew toward each other. The presence of diffusible substances which function in the sexual cycle has since been demonstrated in, for example, *Achlya*³⁻⁸, *Allomyces*^{9,10}, *Ascobolus*¹¹⁻¹³, *Mucor*¹⁴⁻²⁰, *Saccharomyces*²¹⁻²³, and *Neurospora*²⁴⁻²⁷. Varied functions have been attributed to these extracts. In some well studied systems, such as *Achlya* and *Mucor*, chemical substances have been reported at almost all stages of sexual development, each serving to initiate the next stage^{4,17}.

But in spite of all this work relatively little is known about the chemical basis of the incompatibility of heterothallic fungi. Burgeff's work with *Mucor*², was confirmed in both *M. mucedo* and *Blakeslea trispora*²⁸. In *N. crassa*, protoperithecia have been induced to develop into fruiting

bodies, albeit sterile, by filtrates from cultures of the opposite mating type or from mated cultures²⁴. Similar results have been obtained with lipid extracts from single and mated cultures of *N. crassa*²⁷, and from the resulting fruiting bodies a few ascospores were isolated which segregated in an aberrant manner with respect to the mating-type alleles. Unfortunately no tests for other genetic markers were reported.

Table 1 Effect of mycelial extracts from mated cultures on plates of pan 1 and leu 3 assessed by the presence of mature perithecia with spores.

Strain tested	Duration of mating before lysate extraction (h)										
	0	1	6	12	24	48	96	168	240	336	
pan1A	—	—	—	+	+	+	+	+	—	—	
pan1a	—	—	—	+	+	+	+	+	—	—	
leu3A	—	—	—	+	+	+	+	+	—	—	
leu3a	—	—	—	+	+	+	+	+	—	—	

-, No perithecia developed.

+, Perithecia developed.

These and similar reports gave rise to the suggestion that physical contact between compatible mating partners is not essential. Some diffusible substance, termed progamone by Plempel²⁹, induces the sexual cycle in a single strain. If the incompatibility block could thus be totally relieved, then artificial induction of a single strain should be followed by the orderly progression of sexual development. The conclusive demonstration of this induction would be the isolation of resulting ascospores which carry genetic markers from the single parent. This has never been reported, perhaps because the true incompatibility substances have not yet been isolated, or because artificial induction by these substances is followed by aberrant events in the ascogonium leading to strange segregation ratios, as Islam and Weijer²⁷ suggested.

It is significant that previous efforts to overcome incompatibility have relied largely on lipid extracts. Their incomplete success in inducing sexual development might indicate that incompatibility substances, if they exist, are not lipid in nature. Recent suggestions have been that some sex substances are proteinaceous^{23,30,31}. We report here the effect of crude protein extracts on single mating cultures of *N. crassa*. They provide the first conclusive evidence of artificial induction of sexual development in a fungus.

Table 2 Average number of mature perithecia in leu3 plates treated with mycelial extracts.

No. of perithecia per plate	Duration of mating before lysate extraction (h)									
	0	1	6	12	24	48	96	168	240	336
	0	0	0	1,810	3,760	5,840	3,010	2,080	0	0

Figures represent the composites of five plates each.

Emerson strains, obtained from the Fungal Genetics Stock Center, Humboldt, California, provided the extracts. Single cultures were grown for 7 d in large flasks of standard crossing medium³², fertilised with conidia of the opposite mating type and extracted at intervals ranging from 1 to 336 h later (Table 1). Extraction procedures were conducted at 4° C or less. Mycelial mats were homogenised in a Virtis blender with 0.15 M phosphate buffer followed by filtration through cheese cloth, to remove mycelial fragments, and subsequent centrifugation of the filtrate for 30 min at 25,000g. The supernatant was dialysed against 0.05 M phosphate buffer, concentrated by pervaporation and filter sterilised. All extracts were tested to ensure the absence of viable material. Extracts were assayed by adding them to plates of single strain cultures of leu3 (allele R-156) and pan1 (allele 5531) and subsequent 14 d incubation at 25° C. Plates were then inspected for the presence of mature perithecia with spores.

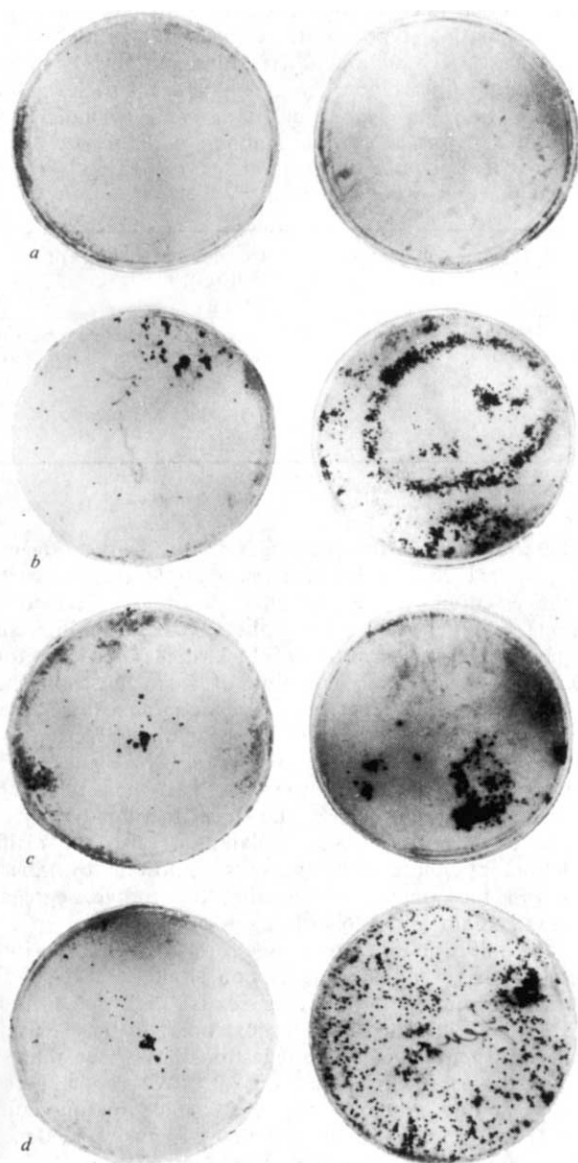


Fig. 1 Effect of mycelial extracts on unmated cultures of *pan1* (left hand side) and *leu3a* (right hand side). *a*, Controls (buffer only added); *b*, with extract from 12 h culture of *Em A* × *Em a*; *c*, with extract from 96 h culture of *Em A* × *Em a*; *d*, with extract from 168 h culture of *Em A* × *Em a*.

The effect of the various mycelial extracts on single cultures of each of the mating types of *leu3* and *pan1* are shown in Tables 1 and 2 and illustrated in Fig. 1. Tetrads were isolated from mature perithecia obtained from eleven different plates (Tables 3 and 4).

The data in Table 1 indicate that the crude extract contains a sex factor capable of inducing the sexual cycle

in a single mating culture of the heterothallic fungus. This substance is present in the mycelial extract of a mated culture; it appears first 12 h after mating and is no longer recoverable 240 h after mating. Preliminary investigations based on lack of activity in medium extracts, suggested that it is not a diffusible substance.

We have demonstrated further that after artificial induction sexual development proceeds normally, culminating in the production of mature ascospores which carry genetic markers from the single parent only (Tables 3 and 4). This is conclusive evidence of artificial induction. The isolation and purification of the active ingredient should ultimately provide the necessary clue to the chemical mechanism of incompatibility. The perithecia produced appear to be morphologically normal but some contain relatively few asci. The incidence of aborted spores (asci with four or eight aborted spores) does not seem to be significantly greater than in normal crosses. Some perithecia contained asci which showed a 4:4 segregation of genetic markers (Table 3). These may have been due to accidental fertilisation by contaminant wild type spores.

Table 4 Tetrad analysis of asci isolated from perithecia present in unmated cultures of *pan1A* treated with mycelial extracts

Plate No.	No. of asci with eight germinated spores	No. of asci with four germinated spores	Segregation patterns of mating-type marker* (A:a)			Segregation patterns of leu marker* (pan ⁻ :pan ⁺)		
			8:0	4:4	4:0	8:0	4:4	4:0
416	4	0	4	0	0	4	0	0
417	3	0	3	0	0	3	0	0
418	6	0	6	0	0	6	0	0
419	4	0	4	0	0	4	0	0
427	2	0	2	0	0	2	0	0

*No. of asci in each class.

The sex inducer, termed erogen B in line with previous terminological suggestions³³, has not yet been characterised chemically. Preliminary tests based on its inactivation by heat and protease but not by nucleases indicate that it is a protein. It is interesting that the extracts from single mating cultures (unfertilised) are inactive in terms of their ability to induce the sexual cycle in strains of the opposite mating type. Thus, erogen B seems to be produced only when both mating types are present in the same culture. Similar results are reported in *B. trispora* and *M. mucedo* where trisporic acid synthesis is initiated only when living mycelia of both mating types are placed in the same culture and stops immediately if one of the partners is removed³⁴. One might predict, from the simplest interpretation of a complementary stimulant model of the chemical mechanism of incompatibility, that the active ingredient, erogen B, from one mating type should successfully induce the opposite mating type. We suggest that the incompatibility mechanism is more complex and that another sex factor, erogen A, is involved. Further details of this work are being published elsewhere.

NORMAN V. VIGFUSSON
RAUL J. CANO*

Department of Biology,
Eastern Washington State College,
Cheney, Washington 99004

*Present address: Department of Microbiology, University of Montana, Missoula, Montana 59801.

Received January 28; revised March 15, 1974.

¹ Raper, J. R., *Am. J. Bot.*, **47**, 794 (1960).

² Burgeff, H., *Bot. Abh. Heft.*, **4**, 5 (1924).

³ Raper, J. R., *Am. Sci.*, **31**, 110 (1951).

⁴ Raper, J. R., *Symp. Soc. exp. Biol.*, **11**, 143 (1957).

Table 3 Tetrad analysis of asci isolated from perithecia present in unmated cultures of *leu3a* treated with mycelial extracts

Plate No.	No. of asci with eight germinated spores	No. of asci with four germinated spores	Segregation patterns of mating-type marker* (a-A)			Segregation patterns of leu marker* (leuΔ-leuΔ)		
			8:0	4:4	4:0	8:0	4:4	4:0
201	6	0	6	0	0	6	0	0
202	4	0	4	0	0	4	0	0
401	3	0	3	0	0	3	0	0
402	3	0	4	0	0	4	0	0
406	4	0	4	0	0	4	0	0
203	0	11	0	0	11	0	0	11
401	10	0	0	10	0	0	10	0

*No. of asci in each class.

- ⁵ Barksdale, A. W., *Am. J. Bot.*, **47**, 14 (1960).
- ⁶ Barksdale, A. W., *Mycologia*, **55**, 627 (1963).
- ⁷ Barksdale, A. W., *Ann. N.Y. Acad. Sci.*, **144**, 313 (1967).
- ⁸ Barksdale, A. W., *Science*, **166**, 831 (1969).
- ⁹ Machlis, L., *Physiol. Pl.*, **11**, 181 (1958).
- ¹⁰ Machlis, L., *Physiol. Pl.*, **11**, 845 (1958).
- ¹¹ Bistis, G., *Am. J. Bot.*, **43**, 389 (1956).
- ¹² Bistis, G., *Am. J. Bot.*, **44**, 436 (1957).
- ¹³ Bistis, G., and Raper, J. R., *Am. J. Bot.*, **50**, 880 (1963).
- ¹⁴ Banbury, G. H., *Nature*, **173**, 499 (1954).
- ¹⁵ Banbury, G. H., *Expl. Bot.*, **6**, 235 (1955).
- ¹⁶ Plempel, M., *Arch. Microbiol.*, **25**, 154 (1957).
- ¹⁷ Plempel, M., *Naturwissenschaften*, **47**, 472 (1960).
- ¹⁸ Plempel, M., and Braunitzer, G., *Z. Naturb.*, **13b**, 302 (1958).
- ¹⁹ Gooday, G. W., *Phytochemistry*, **7**, 2103, (1968).
- ²⁰ Gooday, G. W., *New Phytol.*, **67**, 815, (1968).
- ²¹ Levi, J. D., *Nature*, **177**, 753 (1956).
- ²² Yanagashima, N., *Planta*, **87**, 110 (1969).
- ²³ Yanagashima, N., *Physiol. Pl.*, **24**, 260 (1971).
- ²⁴ Ito, T., *Bot. Mag. Tokyo*, **69**, 369 (1956).
- ²⁵ Howe, Jr., H. B., and Prakash, V., *Can. J. genet. Cytol.*, **11**, 689 (1969).
- ²⁶ Vigfusson, N. V., Walker, D. G., Islam, M. S., and Weijer, J., *Folia Microbiol.*, **16**, 166 (1971).
- ²⁷ Islam, M. S., and Weijer, J., *Folia Microbiol.*, **17**, 316 (1972).
- ²⁸ Ende, H. van den, *J. Bact.*, **96**, 1298 (1968).
- ²⁹ Plempel, M., *Planta*, **59**, 492 (1963).
- ³⁰ Duntze, W., Mackay, V., and Manney, T. R., *Science*, **168**, 1472 (1970).
- ³¹ Ende, H. van den, and Stegwee, D., *Bot. Revs.*, **37**, 22 (1971).
- ³² Westergaard, M., and Mitchell, H. K., *Am. J. Bot.*, **34**, 573 (1947).
- ³³ Machlis, L., *Mycologia*, **64**, 235 (1972).
- ³⁴ Ende, H. van den, Weichmann, A. H. C. A., Reyngoud, D. J., and Hendricks, T., *J. Bact.*, **101**, 423 (1970).

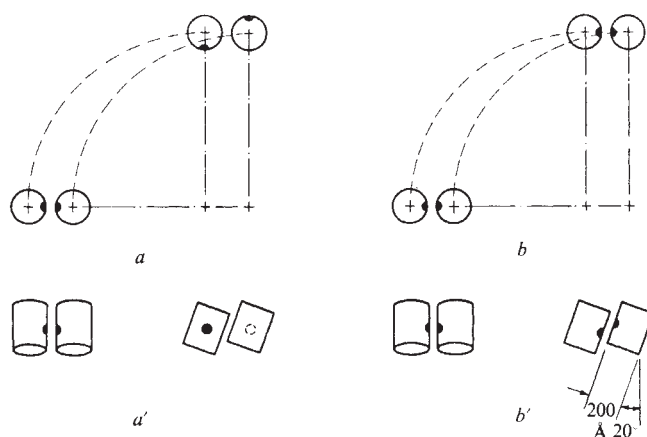


Fig. 1 A schematic view of two adjacent filaments rotating individually (*a* and *a'*) or propagating helical waves (*b* and *b'*) at two successive intervals of time, *a* and *b* are sectional views in a plane normal to the helical axes (+). *a'* and *b'* are elevations of segments of the filaments, projected from *a* and *b* in the plane of the helical axes.

1a and *a'*. The relative motion of the spots on hypothesis *B* is an oscillatory lengthwise rubbing, and we can estimate its amplitude easily by taking the helix angle in Fig. *1b'* as about 20° and the diameter of the filaments as about 200 Å, after Asakura³. Then the extreme displacement of one spot to another is ±70 Å, that is a total throw of 140 Å or about three times the diameter of a flagellin subunit.

Hence, the fact that bivalent flagellar antibodies 'lock' adjacent filaments can only be taken to rule out hypothesis *B* if the antibodies are flexible, and indeed much more flexible than seems likely from what is known about their size and construction.

Now antibodies have a Y-shaped form, with two arms which attach to the host at the tips⁴. From an X-ray crystallographic investigation of the Fab' fragment of an antibody Padlan *et al.*⁵ have determined its overall length to be 80 Å. Thus the length of an arm can be assumed to be about 70 Å. It seems likely that the tips are attached to the host with strong restrictions on the angle of attachment, and so it is difficult to see how such a molecule could stay attached to two points on adjacent flagella executing the large relative motion I have described without having extremely flexible arms.

The arguments of Berg and Anderson therefore do not prove that hypothesis *B* is wrong. Indeed, I believe hypothesis *B* to be correct because the propagation of helical waves in a filament requires only small relative movements between adjacent flagellin subunits, whereas a rigid-body motion of the entire filament would require a very specially constructed bearing. Moreover, as Klug⁶ has pointed out, the problem of propagating a helical wave in a filament is largely subsumed in the problem of assembling a helical filament from identical flagellin subunits, which would still be an outstanding problem on hypothesis *A*.

I thank Dr A. Klug for help.

C. R. CALLADINE

Department of Engineering,
University of Cambridge,
Trumpington Street,
Cambridge CB2 1PZ, UK

Received January 30, 1974.

¹ Berg, H. C., and Anderson, R. A., *Nature*, **245**, 380–382 (1973).

² Di Pierro, J. M., and Doetsch, R. N., *Can. J. Microbiol.*, **14**, 487–489 (1968).

³ Asakura, S., *Adv. Biophys.*, **1**, 99–155 (1970).

⁴ Green, N. M., *Adv. Immun.*, **11**, 1–30 (1969).

⁵ Padlan, E. A., Segal, D. M., Spande, T. F., Davies, D. R., Rudikoff, S., and Potter, M., *Nature new Biol.*, **245**, 165–167 (1973).

⁶ Klug, A., *Symp. int. Soc. Cell Biol.*, **6**, 1–18 (1967).

Bacteria can swim without rotating flagellar filaments

THERE are two simple ways in which the observed screw-like propulsive motion of bacterial flagella can be understood. Either, first, the flagellum is like a rigid corkscrew rotating in a sort of journal bearing in the cell wall (hypothesis *A*) or, second, the flagellum is firmly attached to the cell wall and moves by the propagation of helical waves (hypothesis *B*).

Berg and Anderson¹ argue in favour of hypothesis *A*. Their most positive argument involves the idea that in multi-flagellated bacteria a bivalent antibody firmly attaching itself to two neighbouring flagella would prevent motion according to hypothesis *A* but not on hypothesis *B*. Thus, they interpret the observations of Di Pierro and Doetsch² on the effect of univalent and bivalent flagellar antibodies on both peritrichous and uniflagellar bacteria as support for the 'rotating corkscrew' picture of flagellar motion.

Unfortunately, they have left out of their analysis a simple geometrical factor, which altogether removes from their argument the power to discriminate between the two hypotheses.

Figure 1 (based on Fig. 1 Berg and Anderson¹) shows two cross-sectional views (Fig. *1a* and *b*) along the helical axes of two neighbouring filaments at two successive instants of time according to hypotheses *A* and *B* respectively. In Fig. *1a* the cross sections rotate, whereas in Fig. *1b* they do not. The difference in pattern of relative motion of the spots marked on the surfaces of adjacent filaments is plain.

But now consider Fig. *1a'* and *b'*, which show projected 'elevations' of the situations depicted in Fig. *1a* and *b*. Again the spots are shown but on short segments of the neighbouring filaments in this view. Notice the relative motion of the spots in Fig. *1b'* along the local filament axis, which is not apparent in the view in Fig. *1b* alone. A bivalent antibody attached to the two spots in Fig. *1b* and *b'* could therefore inhibit motion just as strongly as it could in Fig.

Toxicity of piperonyl butoxide to *Boophilus microplus*

SYNERGISM studies with the cattle tick *Boophilus microplus* have been effected by enclosing larvae in packets impregnated with olive oil solutions of the test chemicals and recording mortality after 48 h^{1,2}. In these conditions larvae exposed continuously to 0.4% piperonyl butoxide were apparently unaffected. Subsequently, in studies to determine the sensitivities of the mixed function oxidase systems in organophosphorus (and carbamate) resistant and susceptible strains of *B. microplus*, larvae were immersed in aqueous colloids³ of piperonyl butoxide. Concentrations greater than 0.02% proved to be toxic and an LC₅₀ of 0.044% at 24 h after treatment was determined. This unexpected result aroused interest in the acaricidal potential of piperonyl butoxide. Although it is generally regarded as a nontoxic synergist⁴, some workers have claimed it is toxic to houseflies at fairly high dosage⁵ but others found it to be nontoxic⁶; toxicity to the mite *Acarus*

been shown to inhibit the degradation of carbaryl in laboratory tests on larvae (C. A. S., unpublished data), this type of protective action doubtless contributed to the effectiveness of the spray mixtures. The lethal effect of piperonyl butoxide itself may also have contributed to the enhanced toxicity of the mixtures and it is not certain, therefore, whether piperonyl butoxide acts with carbaryl predominantly as a synergist or mainly as a toxicant participating in some type of joint action.

It seems likely that the toxic action of piperonyl butoxide resulted from inhibition of a mixed function or closely related oxidase, the recognised type of target of piperonyl butoxide¹³. This indicates that the cattle tick is vitally dependent on some part of this system. In support of this we have preliminary evidence that another methylenedioxyphenyl compound sulf-oxide, which inhibits cattle tick mixed function oxidase, is also toxic.

The immediate significance of these findings is that an acaricide which had failed against a resistant strain has been restored to full effectiveness in the field by the addition of a

Table 1 Field control of the Biarra strain of cattle tick with sprays containing piperonyl butoxide or carbaryl or mixtures of the two chemicals

Spray Composition (%)		Cattle per treatment	Survival index* on days† after treatment											Mean 1–21 days
Carbaryl	Piperonyl butoxide		1	2	5	7	9	12	14	16	19	21		
–	0.03	4	99	64	92	132	52	105	86	42	36	38	75	
–	0.3	4	53	28	3	16	33	40	33	14	4	1	23	
–	3.0	4	95	27	0.8	0.7	1	5	4	1	0.4	0.4	14	
0.3	–	2	39	11	3	39	92	16	19	11	9	40	28‡	
0.3	0.03	2	35	22	3	4	33	61	41	24	5	1	23	
0.3	0.3	2	1	0	0	0.7	5	0.7	0.4	0.8	0.8	1	1	
0.3	3.0	2	3	0	0	0.7	4	0.3	0	0	0	0	1	

*Calculated as follows: (Tick count on treated animals/Tick count on control animals) × (Pretreatment count on control animals/Pretreatment count on treated animals) × 100.

On control cattle daily totals of more than 200 semiengorged females were recorded before and after treatment.

†Stages of tick at treatment were: adult females, day 1–8; nymphs, day 9–15; larvae, day 16–21.

‡In similar conditions less than 1% of the acaricide-susceptible Yeerongpilly ticks survived (W. J. R. unpublished data).

siro (formerly *Tyroglyphus farinae*) has been recorded^{7,8}.

We decided that testing of the material alone and mixed with carbaryl, against parasitic stages of the tick in the field, was warranted. The mixture was tested because of the well known synergism of carbaryl and other carbamate insecticides by piperonyl butoxide against resistant houseflies. This synergistic action is believed to be due to piperonyl butoxide reducing the abnormally rapid rates of oxidative degradation of the carbamates by resistant flies⁹. The ticks used in our tests, however, were of the Biarra strain¹⁰, and are resistant to carbaryl because of low sensitivity of their acetylcholinesterase to inhibition by carbaryl, while their detoxication systems are normal¹¹.

Cattle naturally infested with all stages of Biarra ticks were sprayed with aqueous emulsions of piperonyl butoxide formulated with one third its volume of Triton X-100. (*B. microplus* is a one-host tick with a cycle of approximately 21 d, and infested cattle normally carry mixed populations of larvae, nymphs and adults.) Cattle were also sprayed with a commercial wettable powder formulation of carbaryl at 0.3% active ingredient, and with mixtures of carbaryl and the piperonyl butoxide formulation. Counts of semiengorged females 4.5 mm to 8 mm in length were made at intervals up to 21 d after treatment and a survival index was calculated from counts of ticks on untreated control animals¹². The results in Table 1 show that piperonyl butoxide is lethal to all stages of the tick when used at relatively high concentrations. The acaricide carbaryl allowed 28% survival of resistant Biarra ticks compared with 1% survival expected with susceptible Yeerongpilly ticks (W. J. R., unpublished data). With the addition of piperonyl butoxide at concentrations ≥0.3%, however, less than 1% of the resistant strain survived.

As much lower concentrations of piperonyl butoxide have

compound which has no significant mammalian toxicity¹⁴. A more important aspect in the long term could be that from this class of compound a practical acaricide could emerge.

C. A. SCHUNTNER
W. J. ROULSTON
R. H. WHARTON

Division of Entomology,
CSIRO Long Pocket Laboratories,
Meiers Road, Indooroopilly,
Queensland 4068, Australia

Received December 28, 1973; revised March 6, 1974.

¹ Knowles, C. O., and Roulston, W. J., *J. Aust. ent. Soc.*, **11**, 349 (1972).

² Knowles, C. O., and Schuntner, C. A., *J. Aust. ent. Soc.*, (in the press).

³ Roulston, W. J., Schuntner, C. A., and Schnitzerling, H. J., *Aust. J. biol. Sci.*, **19**, 619 (1966).

⁴ Negherbon, W. O., *Handbook of Toxicology*, **3**, 619 (W. B. Saunders Philadelphia, 1959).

⁵ Dove, W. E., *Am. J. trop. Med.*, **27**, 339 (1947).

⁶ Wachs, H., *Science*, **105**, 530 (1947).

⁷ Parkin, E. A., *Pest infest. Res.*, **20** (1950).

⁸ Hope, J. A., *Pest infest. Res.*, **34** (1960).

⁹ Metcalf, R. L., in *The Enzymatic Oxidation of Toxicants* (edit. by Hodgson, E.), 162 (Raleigh, North Carolina State University, 1968).

¹⁰ Roulston, W. J., and Wharton, R. H., *Aust. Vet. J.*, **43**, 129 (1967).

¹¹ Roulston, W. J., Schnitzerling, H. J., and Schuntner, C. A., *Aust. J. biol. Sci.*, **21**, 759 (1968).

¹² Wharton, R. H., Roulston, W. J., Utech, K. B. W., and Kerr, J. D., *Aust. J. agric. Res.*, **21**, 985 (1970).

¹³ Wilkinson, C. F., in *The Enzymatic Oxidation of Toxicants* (edit. by Hodgson, E.), 131 (Raleigh, North Carolina State University, 1968).

¹⁴ Negherbon, W. O., *Handbook of Toxicology*, **3**, 620 (W. B. Saunders, Philadelphia, 1959).

Treehopper (Membracidae) alarm pheromones

ALARM pheromones are characteristic of many species of social Hymenoptera and Isoptera. The suggestion that alarm pheromones occur in gregarious, nonsocial arthropods¹ was confirmed by the report that the heteropteran *Dysdercus intermedius* Distant produces hex-2-en-1-al from its scent glands to repel members of its own species². Aphids, other heteropterans which form aggregates on the plants on which they feed, also produce alarm pheromones. From specialised structures (cornicles) they secrete droplets³ containing volatile pheromones that cause aggregates to disperse when attacked by predators⁴ or parasites⁵. We now report alarm pheromones in three species of myrmecophilous treehoppers (Membracidae), two of which brood the egg mass (Fig. 1a) and all of which form feeding aggregates on their host plants (Fig. 1b). The release of these pheromones is unique in that specialised secretory structures are not involved.

Aggregations of *Vanduzeeia arquata* (Say) on *Robinia pseudoacacia* L., and *Entylia bactriana* (Germar), and *Publilia concava* (Say) on *Ambrosia trifida* were studied in the field and laboratory. Whole treehoppers were crushed on 8-mm diameter filter paper disks which were held by fine forceps 1 cm from the centre of treehopper aggregations. A positive response was recorded when treehoppers moved at least 1 cm from their feeding sites on leaves within 1 min of presentation of the disk. In intra- and interspecific tests, at least five individuals were included in each trial and each combination was tested a minimum of five times. As a control, all test insects were exposed to untreated paper disks before testing with extracts. In field tests, responding individuals first raised their abdomens and expelled a droplet of honeydew from the anus before walking from their feeding sites, often moving to the opposite side of the leaf. Both nymphs and adults of all three species possessed repellent substances and each of the three species responded to compounds produced by the other two species (Table 1). Nymphs were more responsive to their conspecific pheromone than adults. Nearly all responding treehoppers reacted to treated disks within 20 s of initial exposure. No treehoppers responded to the untreated paper disks.

In further tests, filter paper disks treated with three to five drops of honeydew or haemolymph (collected by tibial excision) elicited no response from 63 treehoppers in eight aggregations. Only disks treated with a greenish milky fluid obtained by puncture of the body wall elicited a positive response from 42 of 63 test insects. We found no evidence of an external gland involved in the release of repellent substances. Treehoppers do not respond to cornicle droplets produced by several species of Aphidinae⁶ or to synthetic aphid alarm pheromone, *trans*- β -farnesene, nor do aphids respond to the repellent substances from treehoppers.

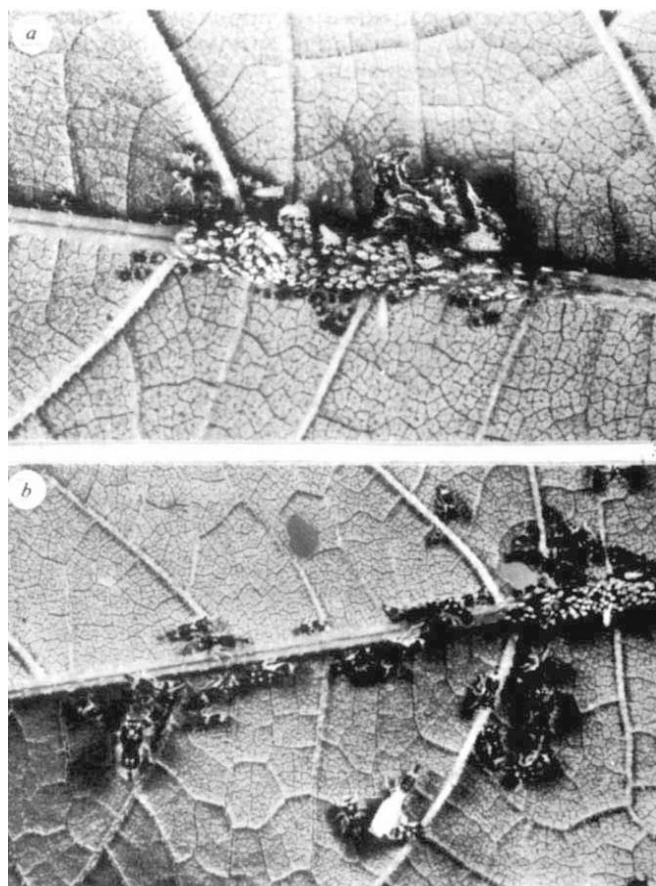


Fig. 1 Adult female *Entylia bactriana* with her egg mass and recently hatched nymphs, (a). Aggregation of middle instar *Publilia concava* near egg mass (b).

Tests designed to determine if coccinellid predators would elicit the alarm response in attacked treehopper aggregations proved frustrating. Both larval and adult beetles usually avoided treehoppers, even when predators were starved 24 h before testing. In only two instances did larval coccinellids attack early instar treehoppers. After puncture of the body wall by the predator's mouth parts, alarm reactions were noted among other treehoppers which then relocated on the opposite side of the leaf.

Our results demonstrate that treehoppers produce alarm pheromone(s) which repel other members of the species. Treehoppers apparently cannot release the pheromone unless the body wall is punctured, such as by an attacking predator. Release of alarm substances in this manner has been described before for sea urchins⁷ and certain species of schooling fishes^{8,9}. It is not certain whether these compounds are specially elaborated pheromones or chemicals which also

Table 1 Interspecific and intraspecific response of treehoppers to alarm pheromones

Species tested	<i>Vanduzeeia</i>		Source of pheromone* <i>Entylia</i>		<i>Publilia</i>	
	Adult	Nymph	Adult	Nymph	Adult	Nymph
<i>Vanduzeeia arquata</i>						
Adult	62.6	53.0	N	N	N	N
Nymph	66.6	65.6	50.0	72.3	51.4	64.1
<i>Entylia bactriana</i>						
Adult	N	N	39.0	24.3	N	N
Nymph	42.8	31.4	45.5	72.9†	27.8	51.5
<i>Publilia concava</i>						
Adult	N	N	N	N	19.4	12.7
Nymph	18.7	60.1	14.6	22.0	16.6	45.9†

* Percentage of individuals responding to pheromone from single treehoppers. From 35–110 individuals in five or more replicates were tested for each combination.

† Significantly more nymphs than adults ($P < 0.05$) responded to pheromone from the species.

N, Not tested.

serve other functions in these organisms. This is the first report of an alarm pheromone in an insect which requires the rupture of the body wall for its release.

L. R. NAULT

Department of Entomology,
Ohio Agricultural Research
and Development Center,
Wooster, Ohio 44691

T. K. WOOD

Division of Biology,
Wilmington College,
Wilmington, Ohio 45177

A. M. GOFF

Department of Biology,
Ashland College,
Ashland, Ohio 44805

Received November 5, 1973; revised March 8, 1974.

¹ Blum, M. S., *A. Rev. Entomol.*, **14**, 57 (1969).

² Calam, D. H., and Youdeowei, A., *Insect Physiol.*, **14**, 1147 (1968).

³ Kislow, C. J., and Edwards, L. J., *Nature*, **235**, 108 (1972).

⁴ Nault, L. R., Edwards, L. J., and Styer, W. E., *Environ. Ent.*, **2**, 101 (1973).

⁵ Goff, A. M., and Nault, L. R., *Environ. Ent.* (in the press).

⁶ Bowers, W. S., Nault, L. R., Webb, R. E., and Dutky, S. R., *Science*, **177**, 1121 (1972).

⁷ Snyder, N., and Snyder, H., *Science*, **168**, 276 (1970).

⁸ von Frisch, K., *Z. Physiol.*, **29**, 46 (1941).

⁹ Thines, G., and Vandenbussche, E., *Anim. Behav.*, **14**, 296 (1966).

New germination inhibitor from *Aegilops ovata* L.

It has been previously reported¹ that the dispersal units of *Aegilops ovata* L. contain a germination inhibitor. Leachates of the hulls inhibited the germination of *Lactuca* achenes. This inhibition was considerably stronger in light than in darkness, an unusual feature, since all germination inhibitors already described inhibit the germination of *Lactuca* achenes in darkness more than in light. We therefore felt it essential to isolate and characterise this specific inhibitor present in *Aegilops ovata*.

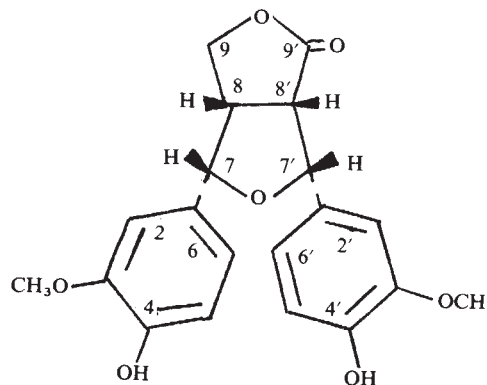
Preliminary investigations yielded a number of compounds namely vanillin, *p*-hydroxybenzaldehyde, ferulic, vanillic, *p*-hydroxybenzoic, *p*-coumaric and syringic acids. The inhibitory activity of these compounds did not, however, account for the initial inhibition observed with the crude extract. Repeated careful chromatography ultimately produced a pure fraction which showed the expected physiological activity. The compound in this fraction, obtained in pure form, was assigned structure (I) on the basis of its NMR, infrared and mass spectra. Structure (I) does not belong to any of the known groups of lignans², and is a monoepoxylignanolate (MEL), since the molecule has an epoxide as well as the five-membered ring lactone.

Table 1 Effect of mono-epoxylignanolate (MEL) on the germination of *Lactuca sativa* cv. "Great Lakes" achenes in light and in darkness at 25° C

Amount of MEL per filter paper (mg)	Light condition	Percentage germination*		
		24 h	48 h	72 h
1	L	0.5±0.5	10±3.7	20±4.2
	D	91.5±1.7	—	—
0	L	95 ±2.4	95.5±2.1	—
	D	95 ±0.6	95 ±0.6	—

L, In light; D, in darkness; *± standard error (4×50 seeds)

The NMR spectrum of compound I shows the following signal pattern: 2,2',5,5',6, and 6' H at δ6.80 (m, 6×H), 7 and 7' H at 5.24 (d, 2×H, *J*=3.0 Hz, 8' H at 3.40 (dd, *J*=9.0 and 3.0 Hz), 8 H at 3.18 (m), 9 H at δ4.1 (dq *J*_{ab}=10.0 Hz, *J*_{ax}=6.0 Hz and *J*_{bx}=4.0 Hz), C(3) and C(3') —OCH₃ groups at δ3.78 (s, 6×H). The two phenolic —OH protons



Compound I

at C(4) and C(4') are at δ5.80 disappearing on exchange with D₂O. The fragmentation pattern in the mass spectrum contributed to the elucidation of the structure. The loss of fragments *m/e* 84 and 85 from the molecular ion peak (*M*⁺ 372) correspond to the five-membered ring lactone. In addition the fragments *m/e* 151 and 137 (base peaks) arise from the loss of $\dot{\text{O}} \equiv \text{C}_6\text{H}_4(\text{OCH}_3)$ (OH) and $\text{H}_2\dot{\text{C}}\text{C}_6\text{H}_3(\text{OCH}_3)$ (OH) respectively. The presence of a five-membered ring lactone in compound I was further substantiated by its infrared spectrum, ν_{max} 1,770 cm⁻¹ characteristic of such a system³.

For the biological assay Whatman No. 1. (diameter 52 mm) filter papers were impregnated with 1 mg quantities of the pure substance. After careful evaporation of the solvent each filter paper was placed in a Petri dish covered with an untreated filter paper and distilled water (2 ml) added. After equilibration over 3 h, 50 achenes of *Lactuca sativa* cv. "Great Lakes" were introduced and the Petri dish kept in a germinator at 25° C either in continuous white light or in darkness. Each test (four replicates) was monitored by a blank (four replicates) in which the lower filter paper was untreated.

The results (Table 1) clearly indicate that the MEL has a unique activity in that it inhibits germination in white light (fluorescent and incandescent) while it is inactive in darkness. This is the first instance to be reported where a compound has such a property. The inhibitory activity of MEL in white light, however, diminishes with time.

D. LAVIE
E. C. LEVY
A. COHEN

Department of Organic Chemistry,
The Weizmann Institute of Science,
Rehovot, Israel

M. EVENARI
Y. GUTTERMANN

Department of Botany,
The Hebrew University of Jerusalem,
Jerusalem, Israel

Received September 5, 1973.

¹ Datta, S. C., Evenari, M., and Gutterman, Y., *Israel J. Bot.*, **19**, 463-483 (1970).

² Freudenberg, K., and Weinges, K., *Tetrahedron*, **15**, 115 (1961).

³ Nakanishi, K., in *Infrared Absorption Spectroscopy*, **44** (Holden-Day, New York, 1964).

Dry deposition of sulphur dioxide on wheat

ABOUT 6 million tonnes of sulphur dioxide (SO_2) are emitted annually into the atmosphere over Britain. Atmospheric concentrations and the quantity of SO_2 exported are determined by the rates of emission, dispersal and removal. Removal as sulphate by rainfall over Britain accounts for about 15% only of annual emission¹. As long ago as 1950 Meetham² suggested that turbulent transfer was an important process for removing SO_2 molecules at the Earth's surface, a process usually referred to as 'dry deposition' even when the surface is wet. Measurements of the dry deposition rates on short grass and water have not been made until recently^{3,4}. From limited evidence for short grass Garland *et al.*¹ suggested that dry deposition over Britain may remove 40% of the SO_2 emitted annually. The surface factors which may control dry deposition rates have not yet been established, however, nor have dry deposition rates on field crops been measured, although it has been suggested that the sulphur supplied in this way may avoid deficiencies on some British soils⁵. Here we report the results of an extensive series of measurements of dry deposition rates on a field of winter wheat and point to factors which may limit the deposition rate.

Table 1 Characteristic measurements of the resistances to deposition of sulphur dioxide on wheat. The SO_2 concentration χ and deposition velocity v_g are referred to $z=1$ m.

Date	Time (GMT)	v_g mm s^{-1}	χ $\mu\text{g m}^{-3}$	$r_a + r_c$ s mm^{-1}	r_c s mm^{-1}	Wet (W) or dry (D)
10/6/1973	10.40	1.5	81	0.65	0.61	D
24/6/1973	11.00	5.4	21	0.18	0.15	D
5/7/1973	18.00	2.0	11	0.49	0.30	D
18/7/1973	19.06	6.4	22	0.16	0	W
24/7/1973	21.00	4.5	26	0.30	0	W
25/7/1973	00.50	5.8	35	0.17	0.06	W
29/7/1973	10.05	1.1	77	0.90	0.85	D
29/7/1973	17.10	3.7	72	0.27	0.23	D
30/7/1973	02.00	7.6	13	0.13	0.04	W
30/7/1973	09.00	4.1	83	0.24	0.20	D
11/8/1973	19.00	4.1	22	0.25	0.10	W
12/8/1973	04.00	4.1	35	0.24	0.05	W

Because the surface is a sink for SO_2 the volume concentration χ decreases towards the surface and the flux F of SO_2 may be determined from measurements of the variation of concentration with height using the standard micrometeorological equation

$$F = K(z) \frac{\partial \chi}{\partial z}$$

where $K(z)$ is the eddy diffusivity for SO_2 at height z , assumed equal to that for momentum. At Sutton Bonington, 10 miles south of Nottingham, values of χ were measured at five heights up to 2 m over a uniform 8 ha field of winter wheat (var Maris Ranger) using the techniques described by Garland *et al.*¹. Eddy diffusivities corrected where necessary for the effects of atmospheric stability⁶ were determined from analysis of wind speed and temperature profiles in the lowest 2 m of the atmosphere.

Deposition rates are often characterised by the velocity of deposition v_g defined by $v_g(z) = \frac{F}{\chi(z)}$ as this quantity is independent of concentration, but to investigate the controlling factors in the deposition process it is convenient to consider the resistance to deposition, $r = \frac{1}{v_g}$ as the sum of an aerodynamic resistance r_a and a surface resistance r_c . The surface resistance is a measure of the affinity of the sur-

face for SO_2 uptake, that is $r_c = 0$ for a perfect sink. Values of r_a were calculated from wind and temperature profiles making allowance for the different processes responsible for mass and momentum transfer at the surface of foliage⁷. The accuracy of the values of v_g depends mainly on the gradient of SO_2 concentration. The chemical analysis was accurate to about $\pm 0.5 \mu\text{g}^{-3} \text{SO}_2$ and the uncertainty in v_g ranged from about $\pm 5\%$ to $\pm 50\%$.

Measurements were made over 1 or 2 h intervals during the period from June 8 until August 12, 1973, which may be arbitrarily divided. Before the last week in July most of the leaves were green and the crop was growing. By the first week in August, the crop was senescent and it was harvested on August 13.

Table 1 shows characteristic results of measurements before and after senescence. The large proportion of the total deposition resistance $r_a + r_c$ contributed by the surface resistance r_c clearly indicates that surface processes limit dry deposition rates in most conditions. When the crop was fully senescent, two regimes were distinguished. When the leaves were dry the SO_2 concentration gradient was generally below the limit of detection, that is $\delta\chi/\delta z < 0.5 \mu\text{g}^{-3} \text{m}^{-1}$. This implies that v_g was very small ($< 0.8 \text{ mm s}^{-1}$) because the surface resistance was large ($> 1.2 \text{ s mm}^{-1}$). There were eight occasions when this occurred but none before August 3. In the late evening the leaves were frequently wetted by dew and the values of r_c decreased substantially. The effect of dew was also observed before senescence, indicating that for wet crops, physiological control of deposition was unimportant. On several occasions when measurements continued sequentially over 24 h the variations in r_c and v_g reveal some interesting features.

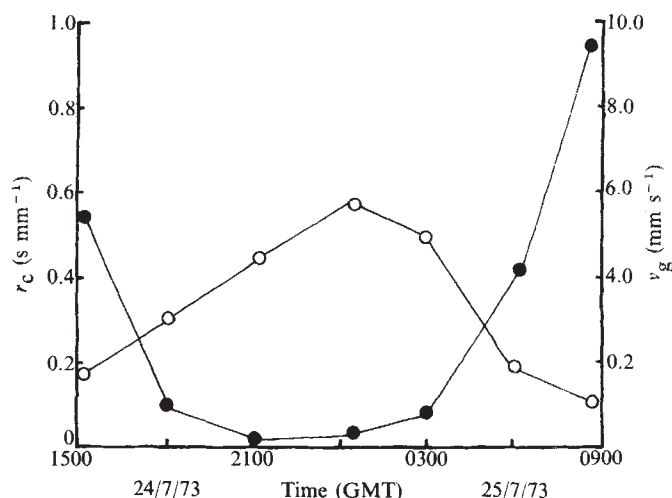


Fig. 1 Variation with time of the deposition velocity v_g (○) and surface resistance r_c (●) for sulphur dioxide on wheat.

Figure 1 shows the diurnal variation of r_c and v_g on July 24. At 1600 h GMT, values of r_c were representative for a dry crop. By 1800 h, dew could be detected on the flag leaves of the crop and the value of r_c was decreasing. As dewfall continued r_c decreased until midnight but then began to increase although dew was still present. The changes in r_c may be explained by a decrease in pH of the dew which decreases the solubility of SO_2 (ref. 8). A similar variation of r_c with time in the presence of dew has been observed in other 24 h experiments. On a night with strong winds and cloud, and consequently no dew, however, r_c remained fairly constant with time.

The mean v_d from 12 occasions when the crop was wet was $4.0 \pm 1.4 \text{ mm s}^{-1}$ and the corresponding mean for r_c was $0.14 \pm 0.12 \text{ s mm}^{-1}$. For 16 occasions when the crop was dry but physiologically active the mean values of v_d and r_c were $3.3 \pm 2.0 \text{ mm s}^{-1}$ and $0.40 \pm 0.26 \text{ s mm}^{-1}$ respectively. The values of v_d and r_c do not differ significantly, but the former is considerably below the value of 8.5 mm s^{-1} for the mean deposition velocity to short grass¹. By extrapolating a mean value of v_d , Garland *et al.*¹ calculated that the annual dry deposition of SO_2 over Britain was 110 kg ha^{-1} , assuming a mean concentration of $40 \mu\text{g m}^{-3}$. If our mean value for v_d (3.6 mm s^{-1}) is more representative of arable crops then with the same assumptions it implies an annual SO_2 deposition of about 47 kg ha^{-1} .

Our results indicate that surface wetness has a major influence on dry deposition rates and that the surface resistance is the controlling step in deposition to vegetated surfaces. A further implication is that in winter, when surfaces are wet for long periods, dry deposition rates of SO_2 may be considerably enhanced.

We thank the Central Electricity Generating Board for a grant and A. Martin, J. L. Monteith, J. Shepherd and staff of the Environmental Sciences Division, Harwell, for assistance and discussions.

D. FOWLER
M. H. UNSWORTH

Department of Physiology and Environmental Studies,
University of Nottingham School of Agriculture,
Sutton Bonington, Loughborough, LE12 5RD, UK.

Received January 25, 1974.

¹ Garland, J. A., Clough, W. S., and Fowler, D., *Nature*, **242**, 256 (1973).

² Meetham, A. R., *Quart. J. R. met. Soc.*, **76**, 359 (1950).

³ Shepherd, J. G., *Atmos. Environ.*, **8**, 69–74 (1974).

⁴ Garland, J. A., Atkins, D. H. F., Readings, C. J., and Caghey, C. J., *Atmos. Environ.*, **8**, 75–9 (1974).

⁵ Cowling, D. W., and Jones, L. H. P., *Soil Sci.*, **110**, 346 (1970).

⁶ Pruitt, W. O., Morgan, D. L., and Lourence, F. J., *Quart. J. R. met. Soc.*, **99**, 370 (1973).

⁷ Thom, A. S., *Quart. J. R. met. Soc.*, **98**, 124 (1972).

⁸ Junge, C., and Ryan, T. G., *Quart. J. R. met. Soc.*, **84**, 46 (1958).

Two spot ladybirds as indicators of intense local air pollution

MOST individuals of the two spot ladybird, *Adalia bipunctata*, may be designated as red with black spots, or as black with red spots. Although intermediate forms are found, they usually constitute less than 1% of the population in Britain. The most commonly occurring black forms are under the control of a single gene and are genetically dominant over the red forms¹. In parts of continental Europe² and in Britain^{3,4}, the frequency of black is highest in industrial and other areas with much atmospheric pollution; up to 97% melanic forms are found in Liverpool and Glasgow. Multiple regression analysis suggests that there is an association of high melanic frequency with smoke, but not with sulphur dioxide⁵, this conclusion is supported by results from Birmingham, where melanic frequencies fell soon after the introduction of extensive smoke control areas, which had only a relatively small effect on sulphur dioxide levels⁶.

Samples previously reported from South Wales³ indicate a consistently low melanic frequency, the maximum being

6% in a sample of 35 individuals from Pontypridd. This is in accord with the occurrence of generally low levels of smoke pollution, in spite of considerable industrialisation⁷.

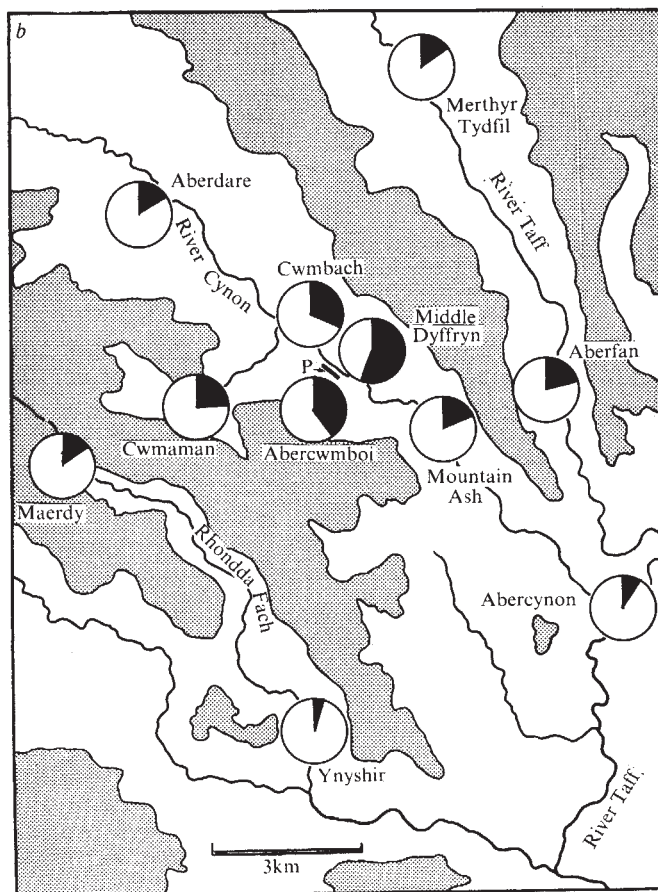
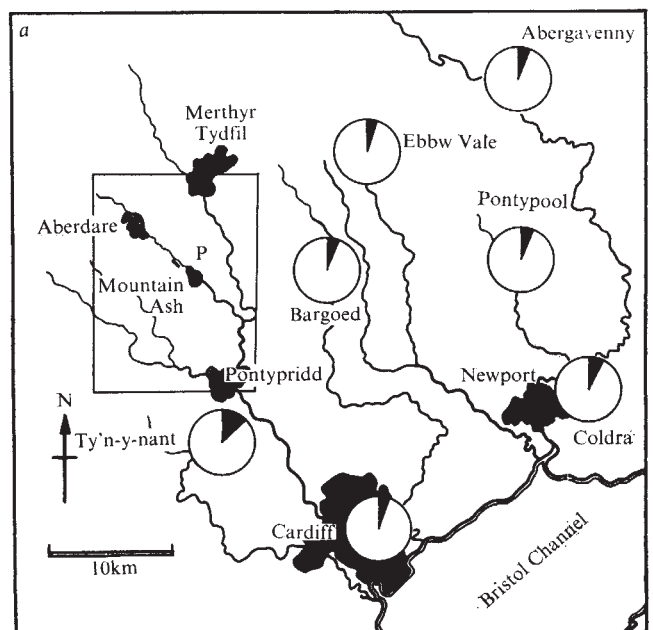


Fig. 1a Map of the eastern valleys of South Wales showing the position of the Phurnacite plant (P), the melanic frequencies at the more distant sampling sites and the area shown in detail in *b*. The relative size of the black segments is proportional to melanic frequency. *b*, Map of the Cynon Valley showing melanic frequencies. For clarity, the Abercwmboi and Middle Dyffryn sites are shown at greater than their true distance (0.3 km) from P. © Ground over 1,000 feet above sea level is stippled.

Table 1 Details of samples of the two spot ladybird, *Adalia bipunctata*, collected in south east Wales in June 1973

	Distance from phurnacite plant (km)	Numbers			% Black
		Red	Black	Total	
Cynon Valley					
Aberdare	4.9	42	7	49	14.3
Cwmaman	2.8	188	60	248	24.2
Cwmbach	1.2	42	20	62	32.3
Abercwmboi	0.3	110	78	188	41.5
Middle Dyffryn	0.3	44	51	95	53.7
Mountain Ash	2.6	22	5	27	18.5
Abercynon	7.6	100	9	109	8.3
Rhondda Fach					
Maerdy	5.5	72	12	84	14.3
Ynysir	7.3	130	6	136	4.4
Taff Valley					
Merthyr Tydfil	6.4	62	11	73	15.1
Aberfan	4.4	93	26	119	21.8
Cardiff	25.9	29	1	30	3.3
Elsewhere					
Ty'n-y-nant	15.1	100	12	112	10.7
Bargoed	12.4	60	3	63	4.8
Ebbw Vale	17.0	69	2	71	2.8
Abergavenny	30.5	49	2	51	3.9
Pontypool	27.4	75	4	79	5.1
Coldra (Newport)	35.2	61	4	65	6.2

A sample of 95 *Adalia bipunctata* was obtained from the Cynon valley in June 1973, however, of which 53.7% were black. The ladybirds were found on willow (*Salix* spp.) and stinging nettle (*Urtica dioica*) over an area of about 75 m × 20 m. The site is in the ruins of the Middle Dyffryn colliery and is 300 m to the east of the ovens of the National Coal Products Phurnacite plant at Abercwmboi, which produces smokeless fuel from coal; it is on the edge of the wood in which the effects of air pollution on the leaves of oak trees were investigated⁸.

To discover how extensive was the area of relatively high melanic frequency, samples were collected from other sites in this part of South Wales (Fig. 1); the results are summarised in Table 1. The sample from the village of Abercwmboi, with 41.5% melanics, was obtained roughly the same distance from the ovens as the first sample, but to the west of them. The difference in frequency between the two samples does not quite reach formal significance; $\chi^2 = 3.78$, $P = 0.052$. The melanic frequency falls steadily with increasing distance from the Phurnacite plant (Fig. 2), and there is no indication that other areas with

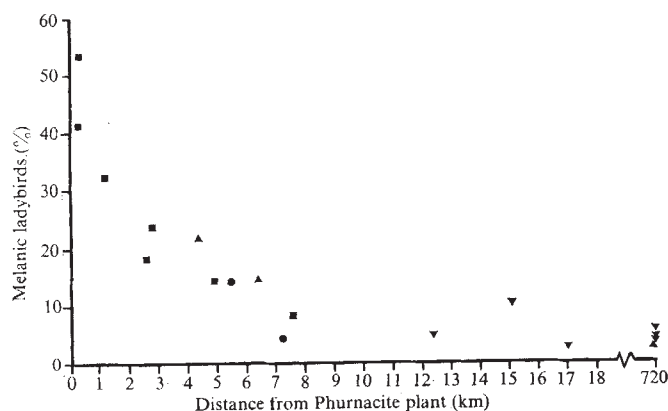


Fig. 2 The relationship between melanic frequency in samples of *Adalia bipunctata* and the distance of the collecting site from the Phurnacite plant at Abercwmboi. ■, Sites in the Cynon valley; ●, sites in the Rhondda Fach; ▲, sites in the Taff valley; ▼, other sites in south east Wales.

locally high frequencies occur in South Wales. The site at Ebbw Vale (2.8% black) is 200 m from large steel and tinplate works, and that at Ty'n-y-nant (10.7% black) is 500 m from coke ovens and chemical works which are, however, much smaller than the Phurnacite plant, but may be responsible for the apparent small increase in melanic frequency.

There is extensive damage to the trees near to the Phurnacite plant, but this is largely confined to those on the east side, the prevailing winds being westerly. Williams *et al.*⁸ concluded however that, although sulphur dioxide was having a marked effect on the trees, high sulphur levels in the leaves resulted from increased epidermal permeability caused by fine particles in the pores of the stomata, rather than from locally high levels of atmospheric sulphur dioxide. Both sulphur dioxide and smoke have lower mean annual levels at a recording point 1.3 km to the ESE of the Phurnacite plant ($44 \mu\text{g m}^{-3}$ and $30 \mu\text{g m}^{-3}$ respectively) than in the urban areas of Mountain Ash ($49 \mu\text{g m}^{-3}$ and $55 \mu\text{g m}^{-3}$) 2.1 km to the south-east, and Aberdare ($60 \mu\text{g m}^{-3}$ and $41 \mu\text{g m}^{-3}$) 4 km to the north-west; but the fallout of particulate matter or grit is very much higher immediately to the east of the Phurnacite plant than elsewhere. In 1967 (the most recent year for which almost complete records are available) total solids at the Middle Dyffryn site were $2.16 \text{ g m}^{-2} \text{ d}^{-1}$, compared with $0.21 \text{ g m}^{-2} \text{ d}^{-1}$ at Abercwmboi (from data supplied by Dr J. Ll. Williams). This high level of particulate matter renders it impossible to use the biological method of smoke and sulphur dioxide assessment⁹ to provide a more detailed comparison of melanic frequencies and air pollution, for I had taken care to avoid proximity to sources of intense pollution. Although I found the reflectance of tree trunks to be the most important single indicator of sulphur dioxide levels over southern Britain, reflectance near the Phurnacite plant is largely influenced by the amount of particulate matter deposited on the surface. The other biological parameters used suggest that both mean smoke and sulphur dioxide levels are lower at the Middle Dyffryn site than in Mountain Ash.

If air pollution from the Phurnacite plant has a direct selective effect on the melanic frequencies, then it seems to be an effect of gases, other than sulphur dioxide, or of the particulate matter comprising grit and smoke. At Aberfan, a melanic frequency of over 20% is found 4.3 km from Middle Dyffryn, and separated from it by high ground on which the insect does not occur; furthermore, the decline in melanic frequency with increasing distance from the Phurnacite plant seems to be the same in samples from the Rhondda Fach and Taff valleys as it is in the Cynon valley (Fig. 2). This suggests that the frequencies are influenced not only by outward gene flow, but also by the Phurnacite plant possibly having an appreciable effect over such distances.

I thank Richard Steward and Dr D. R. Lees for their help with various aspects of this work, and Dr J. Ll. Williams, Medical Officer of Health for Mountain Ash and Aberdare, for the data on pollution levels. The work was supported by a grant from the Nuffield Foundation.

E. R. CREED

Department of Zoology,
University College,
Cardiff CF1 1XL

Received January 30, 1974.

¹ Lus, J. J. *Izv. Byuro Genet.*, **6**, 89–163 (1928).

² Lusis, J. J., *Latv. Ent.*, **4**, 3–29 (1961).

³ Creed, E. R., *Heredity*, **21**, 57–72 (1966).

⁴ Creed, E. R., in *Ecological Genetics and Evolution* (edit. by Creed, E. R.) 134–151 (Blackwell, Oxford and Edinburgh, 1971).

⁵ Lees, D. R., Creed, E. R., and Duckett, J. G., *Heredity*, **30**, 227–232 (1973).

- ⁶ Creed, E. R., *Evolution, Lancaster, Pa.*, **25**, 290-293 (1971).
⁷ Warren Spring Laboratory, *National Survey of Air Pollution* 1961-71, **2** (HMSO, London, 1972).
⁸ Williams, R. J. H., Lloyd, M. M., and Ricks, G. R., *Environ. Pollut.*, **2**, 57-68 (1971).
⁹ Creed, E. R., Lees, D. R., and Duckett, J. G., *Nature*, **244**, 278-280 (1973).

Deltatheroides-like mammals from the Upper Cretaceous of North America

THE family Deltatheridiidae¹ has been redefined to include primitive therian mammals of tribosphenic grade referable to only two genera *Deltatheridium*¹ and *Deltatheroides*², from the Djadokhta and Barun Goyot Formations, of uncertain age within the Upper Cretaceous of Mongolia³. The Deltatheridiidae, long considered Eutheria, although of disputed taxonomic dimensions and phyletic position within the infraclass¹⁻⁵, seem now best classified as therians of metatherian-eutherian grade, but referable to neither Metatheria nor Eutheria². A combination of apparent marsupial-like and placental-like features in the known parts of the dentition and skull of members of the Deltatheridiidae insures that the family will be of continuing interest.

I here record the first recognition of the Deltatheridiidae *sensu* Butler and Kielan-Jaworowska² from territories outside of Asia, an occurrence documented by fossils collected at Upper Cretaceous horizons in the Western Interior of North America. The new deltatheridiids are known only from isolated teeth and tooth fragments, which have been found but rarely in the large samples of North American late Cretaceous mammals in collections. Nevertheless, the detailed resemblances of these fossils to molars of the Asiatic Deltatheridiidae, especially to those of *Deltather-*

*oides cretacicus*¹, make reliable the identifications recorded here. The fossils are: (1) University of Alberta (UA) no. 4085b (see Fig. 1a), a single upper molariform tooth from the late Maestrichtian (Lancian)^{6,7} Upper Edmonton Formation, Alberta, and first described⁸ as a possible deciduous P¹ of the palaeoryctid *Cimolestes magnus*⁹; (2) University of California (Berkeley) Museum of Paleontology no. 46359, a lower molar (see Fig. 1b), and American Museum of Natural History no. 59482, a lower molar trigonid, both from a previously unidentified species occurring in the late Maestrichtian Lance Formation, Wyoming¹⁰; and (3) UA 4248, a previously unidentified lower molar talonid, from the late Campanian (Judithian)^{6,7} Oldman Formation, Alberta. The Lance and Oldman specimens are virtually identical in coronal architecture, other than slightly larger size, to M₂ or M₃ of *Deltatheroides cretacicus* (this species is being redescribed elsewhere by Z. Kielan-Jaworowska). Occlusal relations and comparison with upper molars of the Asiatic deltatheridiids suggest that the tooth from the Upper Edmonton Formation is probably an M¹ from the same or closely related species as are the lower molar specimens.

The close morphological resemblances between the North American and the Asian fossils generate inferences that: (1) the known Asian and North American deltatheridiids diverged relatively recently before their oldest discovered occurrences in the Djadokhta and Oldman Formations; and, as a corollary, (2) migration of the descendant deltatheridiid species from Asia to North America or *vice versa* (and the known evidence does not now permit a choice) took place soon after this divergence. Although these inferences concerning the near contemporaneity of earliest appearances of Asiatic and North American deltatheridiids must remain tentative—at least until more extensive sampling of the local mammalian faunas of North America and East Asia can be completed—they are nevertheless in accord with other evidence that had earlier and independently indicated the improbability of a relatively great Cretaceous age of the Djadokhta mammals¹¹: relict triconodonts and symmetrodonts survived in North America to at least as late as the time of deposition of the early Campanian (Aquilan)^{6,7} Upper Milk River Formation (still before the time of first appearance of North American deltatheridiids), but fossils of neither triconodonts nor symmetrodonts have been found in the deltatheridiid-bearing Djadokhta Formation or in younger Upper Cretaceous rocks in East Asia.

W. Clemens, A. Crompton, Z. Kielan-Jaworowska, M. McKenna and B. Slaughter permitted study of specimens in their care; A. Lindoe photographed the specimens; and D. Krause critically read the manuscript. This work was supported by the National Research Council of Canada and the University of Alberta.

RICHARD C. FOX

Departments of Geology and Zoology,
University of Alberta,
Edmonton, Alberta,
T6G 2E1, Canada

Received March 12, 1974.

- ¹ Gregory, W., and Simpson, G., *Am. Mus. Novit.*, **225**, 1-20 (1926).
² Butler, P., and Kielan-Jaworowska, Z., *Nature*, **245**, 105-106 (1973).
³ Patterson, B., *Fieldiana, Geol.*, **13**, 1-105 (1956).
⁴ Van Valen, L., *Bull. Am. Mus. nat. Hist.*, **132**, 1-126 (1966).
⁵ McKenna, M., Mellett, J., and Szalay, F., *J. Paleont.*, **45**, 441-442 (1971).
⁶ Russell, L., *Contr. Life Sci. Div. R. Ont. Mus.*, **61**, 1-24 (1964).
⁷ Clemens, W., Fox, R., Sloan, R., in *Abst. 1973 Ann. Mtg. geol. Soc. Amer.*, **5**, 577 (1972).
⁸ Lillegraven, J., *Paleont. Contr. Univ. Kansas*, **50**, 1-122 (1969).
⁹ Clemens, W., and Russell, L., in *Vertebrate Paleontology in Alberta*, 32-40 (Univ. Alberta, Edmonton, 1965).
¹⁰ Clemens, W., *Univ. Calif. Publ. geol. Sci.*, **94**, 1-102 (1973).
¹¹ Fox, R., *Nature*, **239**, 170-171 (1972).

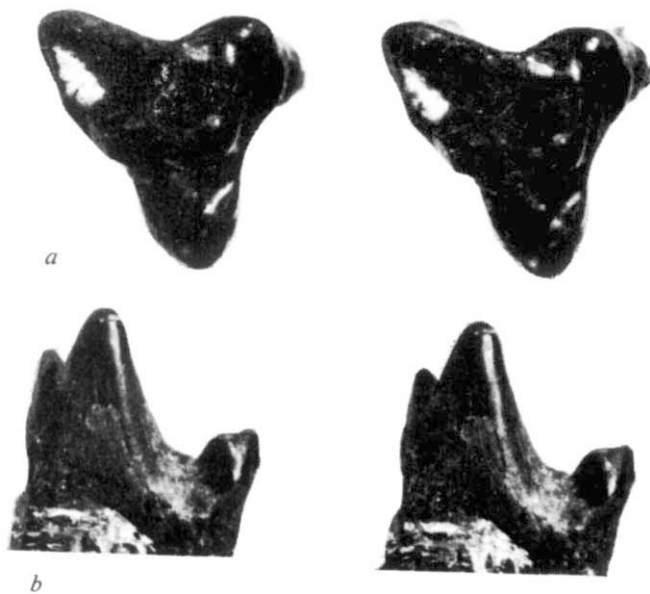


Fig. 1 Stereophoto pairs of isolated molars of cf. *Deltatheroides* sp., Upper Cretaceous, North America. a, UA 4085b, M¹ (?), Upper Edmonton Formation, Alberta, Canada; b, UCMP 46359, lower molar, Lance Formation, Wyoming (× about 7.6).

book reviews

Prehistoric snails

Land Snails in Archaeology, with Special Reference to the British Isles. By John G. Evans. Pp. xii + 436. (Seminar: New York and London, January 1973.) £7.90.

ARCHAEOLOGISTS are becoming increasingly interested in the reconstruction of the environment and ecology of the ancient sites they excavate, and one of the sources of evidence available to them is the careful study of the shells of ancient land snails. These are particularly important in the limestone and chalk regions of Britain where pollen grains do not survive to give direct evidence of the local flora.

This book sets out to give an account of the growth of interest in the study of land snails by archaeologists during the past century, the methods of identification and the results that can be achieved by careful sampling and interpretation.

The possibility of using snail shells as environmental indicators was first suggested by General Pitt-Rivers when he found their remains in the ditch of the iron age hill fort at Cissbury, Sussex, in 1869. Most of the early work on snails in ancient contexts in this country was carried out by A. S. Kennard and B. B. Woodward. Recent developments largely brought about by the author of this book, and recorded in detail in it, include the much more accurate sampling procedures, the interpretation of the environment from the standpoint of both moisture and temperature. "The techniques of snail shell analysis is now refined to the extent that we are able to recognise and differentiate between the effects of climatic, local environmental and anthropogenic influences on ancient snail populations."

Now it is essential that further study be made of the geographical ranges of present day snail populations in this country. Also urgently needed are quantitative data on modern snail populations from habitats whose physical, chemical and biological aspects are fully described. The reconstruction of the past environment depends on establishing the snail fauna of the locality: snail faunas in which 90% of the snails are of one species reflect a severe habitat—salt marsh, ploughed field or sand dune. A wide range of species may be found in well established woodland—upwards of fifty

species is not uncommon, with none of them accounting for more than 15% of the total fauna.

The study of snail faunas ultimately rests on the identification of the remains of the snail shells surviving from the past. The author provides a number of pages of outline drawings of the 200 species involved, including illustrations of immature forms, which should be a splendid guide for those undertaking this identification work. It might have also been helpful to supplement this with photographs both of complete shells, and the most common ways in which they survive as fragments. The species are described in detail, with the environment, distribution and archaeological finds all listed for each species.

Chapter six deals with the ecological groups into which snail faunas may be classified, and may be one of the most interesting sections of the book for those concerned with reconstructing past ecological conditions. They are divided up into shade-loving species, (including the Zonitidae, *Carychium tridentum* and *Discus rotundus* and other woodland species); intermediate or catholic species; open country species (including *Vertigo pygmaea*, *Pupilla muscorum*, *Vallonia costata*, *V. excentrica* and *Helicella itala*); marsh and freshwater slum species. Besides these, four other groups which are not entirely ecological can be distinguished: alien species (introduced into Britain by man at various times since the Bronze Age); burrowing species (*Pomatias elegans* and *Cecilioides acicula* in particular), anthropophobic species, confined to wild habitats in remote woodland (including *Lauria angelica*, *Ena montana*, *Helicodonta obvoluta* and *Limax tenellus*); and synanthropic species which live in man made habitats such as gardens, hedgerows and refuse heaps (for example, *Hygromia striolata*, *Helix aspersa* and *Limax flavus*).

Some snail species are now extinct, which were relatively common in the Boreal period, and thus may be useful for giving some clue for dating the deposits in which they occur, in particular *Vertigo genesii* and *Discus rudersatus*.

The conclusions from work so far are that there was an all-pervading forest cover in Mesolithic and Neolithic times, and that forest clearance at a number of Neolithic sites was fol-

lowed by a period, immediately before the burial of the soil profile, of short turfed grassland. So far snails have not indicated cultivation, except at South Street, Avebury where the ground was ploughed in Beaker times, possibly to create better grazing land. Regeneration of the forests seems to have been prevented for many centuries, probably by grazing, and was never very successful. In the latter half of the first millenium BC the Iron Age farmers began to bring the chalk and limestone soils into more intensive cultivation.

This book marks the beginning of a new phase in the study of ancient land snails, where carefully controlled sampling and analysis can provide the data for the reconstruction of snail faunas, and thus of ancient environments. There are some problems to be overcome, notably the lack of adequate information about some of the modern snail populations, and the fact that some snail species seem to undergo changes in habitat preference (for example *Vallonia costata* and *Helicella itala* which occurred abundantly in prehistoric arable habitats, but are absent from cultivated soils today). It is also possible that some habitats known in the past are now quite extinct.

Land Snails in Archaeology should be on the shelf of every practical archaeologist, and of all those interested in reconstructing the past environments of ancient sites.

JANE M. RENFREW

Mermaid morphology

Morphology of the Sirenia: A Macroscopic and X-ray Atlas of the Osteology of Recent Species. By Hans E. Kaiser. Pp. viii + 76. (Karger: Basel, London and New York, 1974.) Sfr.94; £13.65; \$29.15.

THE true function of this slim but expensive book of photographs is somewhat difficult to discern, though much effort has gone into its production. Here is an ordered series of original photographs, together with a number of positive X-ray photographs, designed to illustrate the macroscopic osteology of the three genera of modern Sirenia. Each photograph is accompanied by a detailed textual description of what is to be seen in that particular picture. But unfortunately a number of the photographs are lacking



CHAPMAN & HALL,
11 New Fetter Lane,
London EC4P 4EE.

Ecological Stability

Edited by M. B. USHER and
M. H. WILLIAMSON

May 1974: 208 pages:

illustrated: hardback: £3.60

The unifying theme of this book is in the analytic investigations of populations, the quantification of population processes and the application of mathematical models to simulate and predict these phenomena. The chapters are based on, but are not identical to, twelve papers given at a 'workshop' meeting at York University in the summer of 1973. The papers fall into four inter-related groups: relations between predator and prey; relations between host and parasite; relations of species within a trophic level; and the effects of special heterogeneity on the stability of species.

CHEMISTRY TEXTBOOK
SERIES

Pericyclic Reactions

G. B. GILL and
M. R. WILLIS

April 1974: 248 pages:

limp cover: £2.95

This book provides a comprehensive introduction to the main theories that have been advanced to explain the stereospecificity of pericyclic reactions. It has been written for use as a text for both undergraduate and postgraduate courses; there are worked examples, formal problems with solutions, and references to the review literature.

The Chemistry of the Non-Metals

P. POWELL and
P. L. TIMMS

April 1974: 274 pages:

illustrated: limp cover: £2.85

This undergraduate level textbook is concerned with the non-metallic elements and the compounds they form with one another. An attempt is made to correlate the chemistry of these compounds as a whole and emphasis is given to discussing common features of structure and bonding within the different classes of compound.

Further information on
these titles and a list of
stockists is available from
the publishers on request.

in clarity, and others are marred by the excessive prominence of the wire ties holding the specimen together. Additionally there are a few line drawings of ossicles, vestigial pelvic bones and so on. But nowhere are scales or measurements indicated except in the initial photograph of the mounted skeletons of *Hydrodamalis*, *Dugong* and *Trichechus* where the overall lengths are stated.

Description is entirely dominant over discussion and comment. The author evidently has little interest in the extraordinary and changing tooth patterns in the dugong and manatee in the course of the life of the individual: pointers on certain pictures simply say "Dentes molares". The non-erupting tusks of the female dugong are nowhere referred to, nor the presence, form and function of the horny buccal pads which are so important in the Sirenia. The work is based on several specimens and not upon series, of skulls, for example.

G. C. L. BERTRAM

International eel

Der Aal: Biologie und Fischerei. By Friedrich-Wilhelm Tesch. Pp. 306. (Parey: Hamburg and Berlin, 1973.) DM 78.

THE physiology and migrations of eels raise fundamental and controversial research questions. This book will give a further impetus to work in this field, for it surveys the entire literature (about 900 references) dealing with the Atlantic as well as the Indopacific species of Anguillidae.

Two-thirds of the book are devoted to zoological aspects, with an especially thorough discussion of anatomy, variation, taxonomy, life history, feeding and migrations. The separation of two Atlantic species, the American, *Anguilla rostrata* and the European, *A. anguilla* is defended against the background of a recent controversy. Dr Tesch attributes their geographical separation to the time required for the transition from larva to elver. The European species requires for this three years, so that larvae brought prematurely near American shores will not be ready to respond by movement to freshwater. American larvae become elvers in one year and any transported eastwards will perish as they will not be near the coast at the appropriate ontogenetic stage.

The chapters on environmental influences on productivity of inland waters, growth, behaviour and even sex determination and sex ratios are particularly detailed. Certain current biochemical studies, however, are not discussed in sufficient detail (and Sick's haemoglobin studies were electropho-

insects in flight

WERNER NACHTIGALL

The current knowledge and latest research findings in the biophysics of insect flight are combined in this book and explained in a way that is clear to the general reader without neglecting accuracy of detail. The author describes the fascinating inventiveness of Nature, revealing the multiplicity and intricacy of her designs. The book contains a large number of illustrations and unique photographs. £5.50

George Allen & Unwin

retic not chromatographic, page 41).

In addition to the main body of information, minor but fascinating issues are not forgotten: the methods and difficulties of determining age and sex of specimens, the poison in the uncooked eel blood, the survival of a specimen with very little haemoglobin, the swimming ability of the larva, and so on.

The last third of the book deals expertly with methods of fishing and other related issues. The increasing economic importance of the eel is clearly demonstrated. Characteristically, for our age, the collection of elvers for food and the impediments to elver immigration presented by technical works, are leading in Japan to scarcity of eels. This is met by fish farming, and there are over 1,200 eel farms in that country. The methods used in these are described.

This is an excellent book, impeccably produced and with very helpful illustrations and tables. I hope that it will be translated into English.

E. M. PANTELOURIS

Erratum

The price of *Computational Physics* by David Potter (reviewed in *Nature* 248, 295; 1974) is £6.95 and not as stated, and there is no paperback edition.

announcements

International meetings

June 2–6, **Radiation Protection: Philosophy and Implementation** (Symposium Organiser, Room 1324, CEGB, Sudbury House, 15, Newgate Street, London EC1A 7AU)

June 3–5, **A festive International Exposition for the Minicomputer Industry** (MINIFEST 74 Secretary, Polytechnic of Central London, 115, New Cavendish Street, London W1M 8JS)

June 3–5, **Applications of Marine Geodesy** (G. W. Lennon, Institute of Coastal Oceanography and Tides, Bidston Observatory, Birkenhead, Cheshire, L43 7RA, UK)

June 3–6, **25th Annual Meeting of the Tissue Culture Association** (Dr Vincent J. Cristofalo, Chairman, TCA Publicity Committee, The Wistar Institute, 36th Street at Spruce, Philadelphia, Pennsylvania 19174)

June 3–7, **International Symposium on N₂ Fixation** (Dr William E. Newton, Charles F. Kettering Research Laboratory, 150, East South College Street, Yellow Springs, Ohio 45387)

June 4, **Using Minicomputers and Programmable Calculators for Stress Analysis and Design** (The Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 8QX)

June 4–7, **Minerals and the Environment** (Institute of Mining and Metallurgy, 44, Portland Place, London W1N 4BR)

June 6–7, **7th Geodesy/Solid-Earth and Ocean Physics (GEOP) Research Conference** (American Geophysical Union, 1707, L Street N.W., Washington DC 20036)

June 7, **Symposium on Mathematical Economics** (Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY, UK)

June 10–13, **8th Annual Conference on Trace Substances in Environmental Health** (Dr Delbert Hemphill, 426, Clark Hall, University of Missouri, Columbia, Missouri 65201)

June 10–14, **Haploids in Higher Plants: Advances and Potential** (University of Guelph, Ontario, Canada)

June 11, **Symposium on Mathematics in Educational Planning** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY, UK)

June 11–14, **The Electrodynamics of Substorms and Magnetic Storms** (R. A. Hoffman (Chairman), NASA, Code 646, GSFC, Greenbelt, Maryland 20771)

June 11–14, **8th International Congress for Noise Abatement** (Secretariat Pro Aqua-Pro Vita, ACIB, P.O. Box, CH-4021 Basle, Switzerland)

June 12, **Changing your Computer** (Registration Secretary, BIS-Brandon Training, 80, Blackfriars Road, London SE1 8HB)

June 12, **Glass: An Engineering Material** (The Meetings Secretary, Institution of Metallurgists, Northway House, Whetstone, London N20 9LW)

June 12–14, **International Conference on Submillimeter Waves and their Applications** (K. J. Button, MIT National Magnet Laboratory, Cambridge, Massachusetts 02139)

June 12–15, **Analysis of Lipids and Lipoproteins** (1974 AOCS Lipids Conference, American Oil Chemists' Society, 508, South Sixth Street, Champaign, Illinois 61820)

June 14–22, **17th International Seed Testing Congress** (ISTA Secretariat, Box 68, N 1432 AS-NLH Norway)

June 16–20, **11th Informal Conference on Photochemistry Honoring Professor Francis E. Blacet** (T. W. Martin, Conference Chairman, Box 1506/B, Vanderbilt University, Nashville, Tennessee 37235)

June 17–19, **Velikovsky and the Recent History of the Solar System** (Stephen L. Talbott, Editor *Pensee* Magazine, P.O. Box 414, Portland, Oregon 97207)

June 17–21, **5th International Liquid Crystal Conference** (Conference Secretariat, c/o Stockholm Convention Bureau, Strandvagen 7c, S 114 56 Stockholm, Sweden)

June 19, **26th Annual CIBA Foundation Lecture** (Personal Assistant to the Director, The Ciba Foundation, 41, Portland Place, London W1N 4BN)

June 21–26, **3rd International Congress of Plant Tissue and Cell Culture** (The Secretariat, IAPTC Congress, Botanical Laboratories, University of Leicester, Leicester, LE1 7RH, UK)

June 22–27, **Congress Tertius Societatis Radiologicae Europaeae** (The British Institute of Radiology, 32 Welbeck Street, London W1M 7PG)

June 23–26, **1st International Meeting of the Society "Environment and Health"** (The Secretariat, The National Research Foundation, 48, Vassileos Constantinou Avenue, Athens 501/1, Greece)

June 23–30, **Workshop on Medical Ethics** (Robert M. Veatch, Associate for Medical Ethics, Institute of Society, Ethics and the Life Sciences, 623, Warburton Avenue, Hastings-on-Hudson, New York 10706)

June 24–26, **2nd International Symposium on Mass Spectrometry in Biochemistry and Medicine** (Secretariat c/o Instituto di Ricerche Farmacologiche "Mario Negri" 20157 Milan, Via Eritea, 62, Italy)

June 24–29, **Paris 1974 Immuno-Oncology Week** (Secretariat of the Institut de Cancerologie et D'Immunogenetique, 14, Avenue Paul-Vaillant-Couturier 94800 Villejuif, France)

June 24–28, **Recent Advances in the Assessment of the Health Effects of Environmental Pollution** (The Secretariat, Health Protection Directorate, 29, Rue Aldringen Luxembourg (Grand Duchy))

June 24–28, **Enzymes and their uses in Analysis and Clinical Diagnosis** (Director of the Summer Session, Room E19-356, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139)

June 24–28, **2nd International Symposium on Sex Education** (The Organising Committee, Second International Symposium on Sex Education, P.O.B. 16271, Tel Aviv, Israel)

June 24–28, **9th International Symposium on the Chemistry of Natural Products** (Executive Secretary, 9th International Symposium on the Chemistry of Natural Products, c/o National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada)

June 25–July 6, **10th International Symposium on Mathematical Geophysics** (Dr J. A. Hudson, Department of Applied Mathematics and Theoretical Physics, Silver Street, Cambridge, UK)

June 27–29, **Gene Regulation in Mammals** (Dr Andrew A. Kandutsch, The Jackson Laboratory, Bar Harbour, Maine 04609)

July 1, **Automated Sequencing for Peptides and Proteins** (Chairman, J. I. Harris, MRC Cambridge, UK)

July 1–3, **549th Biochemical Society Meeting** (The Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP)

July 1–5, **6th Symposium on Organic Sulphur Chemistry** (The Secretariat, School of Physical and Molecular Sciences, University College of North Wales, Bangor, LL57 2UW, UK)

July 1–6, **Land use and Society** (The Secretary, S₂A₃ Congress, c/o Rhodes University, Grahamstown, South Africa)

July, 2–4, **The Emergence of Science in Western Europe in Different National Contexts** (The British Society for the History of Science, 47 Belgrave Square, London SW1X 8QX)

July 7–12, **9th International Congress of Neuropsychopharmacology** (Professor J. R. Boissier, General Secretary, Organising Committee, Unite de Neuropsychopharmacology, 2 Rue d'Alesia, 75014 Paris, France)

July 8–10, **Recent Advances in NMR Spectroscopy** (Miss J. M. Usher, The Chemical Society, Burlington House, London W1V 0BN)

July 8–11, **2nd International Conference on the Biology and Pharmacology of Cyclic AMP** (Dr G. I. Drummond, Department of Pharmacology, Faculty of Medicine, University of British Columbia, Vancouver 8, Canada)

July 8–12, **International Dynamic Mass Spectrometer Symposium** (Dr D. Price, Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, Lancashire, UK)

July 8–12, **Micro 74** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA, UK)

July 9–12, **Kinetics of Chemical Reactions in Heterogeneous Systems** (Dr Trojanowsky, Secrétaire général, Sociéte de Chimie physique, 10 rue Vauquelin, 75231 Paris Cedex 05, France)

July 9–12, **Interrelation of Structure, Properties and Applications of Polymers** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

July 10–12, **Environmental Pollution: Mutation and Selection** (Professor M. A. Ferguson-Smith, Institute of Genetics, Church Street, Glasgow G11 5JS, UK)

July 11–12, **Recent Advances in Vibrational Spectroscopy** (Miss J. M. Usher, The Chemical Society, Burlington House, London W1V 0BN)

July 11–12, **Trace Elements and Nutrition** (Dr J. D. Sutton, Hon. Programmes Secretary, Nutrition Society, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, UK)

July 12–16, **8th International Congress on Animal Reproduction and Artificial Insemination** (Professor Stefan Wierzbowski, 32-083 BALICE/Krakow, Poland, Institute of Zootechnics, Department of Animal Reproduction and AI)

July 14–20, **5th International Congress of Radiation Research** (Dr W. K. Sinclair, Secretary-General, Argonne National Laboratory, Argonne, Illinois 60439, USA)

July 15, **Workshop Course on Gradient Centrifugation** (Dr E. Reid, Wolfson Bioanalytical Centre, University of Surrey, Guildford GU2 5XH, UK)

July 15–19, **Dynamic Studies with Radioisotopes in Clinical Medicine and Research** (International Atomic Energy Agency, Karntner Ring 11-13, P.O. Box 590, A-1011 Vienna, Austria)

July 15–19, **Course in Modern Microscopy** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA, UK)

July 15–19, **Centenary Celebrations of the Society for Analytical Chemistry** (The Secretary, Society for Analytical Chemistry, 9/10 Savile Row, London W1X 1AF)

July 16–19, **John Innes Symposium: Modifications of the Information contents of Plant Cells** (Miss Justine Speed, Symposium Secretary, John Innes Institute, Norwich NOR 70F, UK)

July 17, **Society for Drug Research, Anti-Obesity Drugs** (Dr J. R. Cavalla, c/o John Wyeth and Brother Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire, UK)

July 21–26, **3rd International Congress of Plant Tissue and Cell Culture** (Secretariat, Third International Congress of Plant Tissue and Cell Culture, Botanical Laboratories, University of Leicester, Leicester LE1 7RH, UK)

July 21–27, **The Applications of Heterogeneous Catalysis** (Centre for Extension Studies, University of Technology, Loughborough, Leics., LE11 3TU, UK)

July 22–26, **Enzyme and Fermentation Biotechnology Course** (The Assistant Secretary, The Society of Chemical Industry, 14 Belgrave Square, London SW1)

July 22–26, **Course in Photomicrography** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA, UK)

July 22–26, **International Conference on Urban Development Models** (Mrs M. Houston, Land Use and Built Form Studies, University of Cambridge, 16 Brooklands Avenue, Cambridge, UK)

July 22–August 9, **Origin and Abundance of the Chemical Elements** (Professor Martin Rees, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK)

July 23–31, **8th International Congress on Acoustics** (The Administrative Secretary, Eighth International Congress on Acoustics, 47 Belgrave Square, London SW1X 8QX, UK)

July 25–27, **29th Annual Calorimetry Conference** (Harry Watts, Research and Development Department, Dow Chemical of Canada, Limited, P.O. Box 1012, Sarnia, Ontario N7T 7K7, Canada)

July 29–August 9, **Energy: A Unified View** (Director of the Summer Session, MIT, Room E19-356, Cambridge, Massachusetts 02139, USA)

August 1–2, **International Symposium on Microwave Acoustics** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

August 2–8, **International Committee on Avian Anatomical Nomenclature** (Dr John McLelland, Secretary of ICAAN Department of Anatomy, Royal School of Veterinary Studies, Edinburgh EH9 1QH, Scotland, UK)

Erratum

In the article "Speeding up astronomical photography" (*Nature*, **249**, 22; 1974) by P. M. Corben, V. C. Reddish and M. E. Sim, the letters *a* and *b* on Fig. 2 were unfortunately interchanged during preparation of the illustrations for printing.